THE
AMERICAN NATURALIST

VOL. LXXI *July–August, 1937* No. 735

THE EFFECT OF VARIATION ON FITNESS

DR. J. B. S. HALDANE
JOHN INNES HORTICULTURAL INSTITUTION

THERE is good reason to believe, with Darwin, that natural selection has played a very important part in evolution. The great interest of the evolutionary process has tended to divert attention from the action of natural selection in stabilizing species in their existing monomorphic or polymorphic facies. Yet this latter phenomenon is easily observable.

On the other hand, the evolutionary process is exceedingly slow. Forms usually change little in 100,000 years. Now Haldane (1924) showed that a dominant character causing an increase of 0.1 per cent. in the fitness of its carriers would increase from a frequency of .001 per cent. to one of 99 per cent. in a random mating population in 23,400 generations, and somewhat more rapidly in an inbred population; in fact, on a geological time scale, almost explosively. But a difference of fitness of this magnitude could not be detected. In order that an observed viability difference of 0.1 per cent. should exceed twice its standard error, we should have to observe at least sixteen million individuals. To detect so small a difference in fertility we should have to count their progeny.

It may be possible to observe evolution by natural selection in a species which is adapting itself to a new environment. In other cases we can very rarely hope to notice evolutionary changes within a human lifetime. From the standpoint of an individual human observer species may be regarded as almost in equilibrium. Our only reason to

hope for observable evolution is that owing to glaciation, agriculture, fishing and industry, the balance of nature has recently been upset in a manner probably without precedent in our planet's history; and hence on the Darwinian theory we should expect that evolution was proceeding with extreme and abnormal speed.

However, in what follows we shall deal entirely with populations in equilibrium. Every species observed with sufficient care has been found to include members less fit than the average and whose lack of fitness is heritable. Their number in a sufficiently large population is approximately constant, and in spite of selection does not diminish, either because the genes or chromosomal abnormalities responsible for them are continually being replenished as the result of mutation, or because they are advantageous in a different combination. We shall here discuss the effect of such deleterious genes on the fitness of the species.

We must first define fitness. This is easiest in a hermaphrodite organism. We can say that the fitness of any particular genotype (or group of genotypes) is half the mean number of progeny left by an individual of that genotype. Progeny due to self-fertilization are counted twice over. Certain conventions are necessary. Obviously, individuals must be counted at the same stage of the life-cycle, *e.g.*, at birth or maturity. Also when determining our average fitness we must take arithmetic means in space, but geometric means in time. Thus if two organisms have 18 and 2 progeny, respectively, their mean fitness is 5, not 3. And in an organism with two generations per year, the autumn generation being 5 times as numerous as the spring population, the mean fitness of the spring population is 5, of the autumn population 1/5, their mean being unity, the geometric mean, not 2.6, the arithmetic mean.

If we take the generation as our unit of time, the natural logarithm of the fitness is the Malthusian parameter as defined by Fisher (1930). Fisher took the year

as his unit; and where generations are not sharply defined an astronomical unit is preferable to a biological. In such a case the precise mathematical theory (Norton 1928, Haldane 1926) is rather complicated. However, our general conclusions are unaffected by the complications.

In a bisexual organism a correction must be made for the sex-ratio. And in a polymorphic population with several exogamous genotypes (*e.g.*, a plant with trimorphic heterostylism) the matter is further complicated. An example of the necessary computations is given by Fisher (1935) in the case of *Lythrum Salicaria*. However, unless a mutant gene or chromosomal abnormality affects the sex-ratio no complications of this type occur in the case of animals.

It must be emphasized that high fitness of a particular genotype as here defined does not ensure its increase, even in the absence of back mutation. Thus a type carrying an extra chromosome fragment may be both more viable and more fertile than the normal but may nevertheless tend to die out because the fragment is contributed to less than half the viable gametes. Or a gene advantageous to the zygotes may be handicapped in pollen-tube competition with an allelomorph. However, most characters are determined by genes. And since as a result of normal mendelian segregation genes do not increase or diminish in number in a population, increase of fitness will cause a spread of the genes which determine it, other things being equal. Finally, fitness as defined above may be a function of the mating system in the population considered, and will be altered by changes in the degree either of inbreeding or of assortative mating, as well as by Darwinian sexual selection. For example, the fitness of an unfit type is generally lowered by inbreeding, because it is more likely to find an unfit mate than in an outbred population.

It is clear that the mean fitness of all members of a species must always be very close to unity, if we average over any length of time. If the fitness were 1.01 the population would increase 20,959 times in 1,000 genera-

tions. In almost all species the mean fitness over 1,000 generations must vary from unity by far less than one per cent. But in any species some genotypes have a fitness less than unity, ranging to zero in the case of lethal genes and genes causing complete sterility. So it is clear that the fitness of the standard type containing no deleterious genes must exceed unity. A population composed of such a type would of course increase until, owing to its pressure on the means of subsistence, the fitness was again reduced to unity.

At any gene locus in a population there are a number of possible conditions which may be listed as follows:

1. *Equilibrium*
   a. Equilibrium between genes whose effect on fitness is of the order of their mutation rates or smaller.
   b. Equilibrium due to greater fitness of heterozygotes than homozygotes.
   c. Equilibrium due to the constant production by mutation of genes lowering fitness and thus eliminated by selection.
   d. Equilibrium due to exogamy.
   e. Equilibrium due to inhomogeneous environment, etc.
2. *States of Change*
   a. Decrease in frequency of a gene lowering fitness.
   b. Increase in frequency of a gene lowering fitness.

There will also be equilibrium due to a combination of causes. *E.g.,* heterozygous forms such as the thrum primrose which are kept in existence by exogamy may also be *per se* fitter than homozygotes. And equilibrium 1(a) shades off into 1(b) and 1(c) imperceptibly.

Equilibria of types a, b, d and e may give rise to polymorphism. Type c will give a number of abnormalities, each much rarer than the normal type, but the population as a whole will be monomorphic, except for sexuality and results of equilibrium due to other causes. We shall now investigate equilibria due to this cause.

New genes constantly arise by mutation. It is well known that most mutant types are less fit than the normal in the wild state, even if they are more so in abnormal conditions such as domestication. It is *a priori* obvious that this must be so. For a gene with any appreciable mutation

frequency must have appeared many times in the past (except perhaps in species such as the elephants or *Sequoia gigantea* with very few individuals). Hence if it produced an increase of fitness, it would already have spread through the population.

It is, however, hardly justifiable to describe such abnormal genes as pathological in all cases, although they may be so. In the first place, they may lead to increased fitness in a different environment. Thus Sax (1926) found that bean plants from recessive white seeds were more fertile than their sisters from colored seeds in good years, less so in bad years when the environment was presumably more like that of wild plants. Secondly, several abnormal genes together may increase fitness, as Haldane (1931) and Wright (1931) have pointed out. If so the standard or normal type is not the fittest type in the population. Nevertheless, the fittest type will not spread, since the abnormal genes generally occur one at a time, thus lowering fitness, and only rarely all together.

It is at once clear that in equilibrium such abnormal genes are wiped out by natural selection at exactly the same rate as they are produced by mutation. It does not matter whether the gene is lethal or almost harmless. In the first case, every individual carrying it, or if it is recessive, every individual homozygous for it, is wiped out. In the second the viability or fertility of such individuals may only be reduced by one-thousandth. In either case, however, the loss of fitness to the species depends entirely on the mutation rate and not at all on the effect of the gene upon the fitness of the individual carrying it, provided this is large enough to keep the gene rare. This conclusion will be proved in detail for the four individual cases.

Dominant Autosomal Abnormal Genes

Consider a normal gene which mutates to a dominant allelomorph with frequency μ per locus per generation. The situation is precisely the same with regard to a cytological abnormality, such as duplication, deficiency or in-

version, provided it lowers the fitness in the heterozygous condition and exhibits mendelian inheritance. If the dominant gene lowers the fitness appreciably it will be rare, like most human dominant abnormalities, and homozygotes will be so rare as to be negligible. Let N be the number of the population, x the frequency of the mutant type and f its fitness. f is of course an average value and must be less than unity, even if the mutant gene increases fitness in certain genetic combinations or in certain environments.

Then in each generation the number of abnormal genes is increased by $(2-x)\mu N$ as the result of mutation. It is diminished by $x(1-f)N$ as the result of selection. As there is equilibrium $(2-x)\mu N = x(1-f)N$, so

$$x = \frac{2\mu}{1-f+\mu}$$

Since μ is generally a small quantity of the order of 10^{-6} or less, $x = \frac{2\mu}{1-f}$ approximately. Thus if $\mu = 10^{-6}$ and $f = 0$, $x = 2 \times 10^{-6}$. If $f = .999$, $x = 2 \times 10^{-3}$. The gene remains rare until $1-f$ is nearly as small as μ. The loss of fitness to the species due to the gene is $x(1-f), = 2\mu - \frac{2\mu^2}{1-f+\mu}$, or 2μ very approximately. This is independent of the value of f.

When a species is highly inbred, even a rare dominant will often appear in the homozygous condition. This will be particularly the case in a species which is predominantly self-fertilized. Let us suppose that the fitnesses of the heterozygote and homozygote are f_1 and f_2, respectively, their frequencies y and x, respectively. Then from the conditions of equilibrium

$$y = 2\mu(1-y-x) - \mu y + \tfrac{1}{2} f_1 y$$
$$x = \mu y + f_2 x + \tfrac{1}{2} f_1 y$$

Hence, neglecting small quantities,

$$x = \frac{f_1 \mu}{(2-f_1)(1-f_2)} \qquad y = \frac{4\mu}{2-f_1}$$

And the loss of fitness to the species is $(1-f_1)y +$

$(1-f_2)x$, or $\frac{(4-3f_1)\mu}{2-f_1}$ which lies between μ and 2μ, as is otherwise obvious. Similar expressions can be obtained for less intense degrees of inbreeding.

Sex-linked Abnormal Genes

Again let the frequency of mutation be μ, the frequency of the gene in the population x, and the fitness in females (supposed to be homogametic as in mammals) f_1, in males f_2. Assuming a sex-ratio of equality, there are $\frac{3}{2}N$ loci in the whole population. Of the $\frac{3}{2}Nx$ abnormal genes Nx will be in females, $\frac{1}{2}Nx$ in males. Hence the mutation rate $(\frac{3}{2}-x)\mu N$ must be equal to the selection rate $Nx\left(\frac{1-2f_1+f_2}{3}\right)$

Hence

$$x=\frac{3\mu}{2\left(\frac{1-2f_1+f_2}{3}\right)}$$

The loss of fitness is

$$x\left(\frac{1-2f_1+f_2}{3}\right)$$

or $\frac{3}{2}\mu$. This is true whether the gene is dominant, recessive or intermediate, provided only that f_1 or f_2 is large enough in comparison with μ to keep it rare. A fuller treatment of this question has been given by Haldane (1935) in a discussion of the origin of haemophilia by mutation.

Of course the actual state of affairs in the population depends on the degree of dominance of the gene. A recessive gene will manifest itself in males only. A dominant gene will appear in about twice as many females as males. A gene of intermediate dominance will appear in some members of each sex. But so long as it remains so rare that homozygous females are not found, one gene is de-

stroyed for each individual eliminated by natural selection, just as with a rare autosomal dominant.

RECESSIVE AUTOSOMAL GENES

Here let μ, f and x have their former values, and let $2y$ be the frequency of heterozygotes. Then the number of abnormal genes produced per generation is $2(1-x-y)\mu N$, the number eliminated $2x(1-f)N$. The factor 2 arises in the latter case because the destruction of a homozygote involves that of two abnormal genes. Provided that x and y are small, $x=\frac{\mu}{1-f}$ and the loss of fitness to the species is μ. In a random mating population, if p be the frequency of the recessive gene, $x=p^2$, $y=2p$, so $(1-p)\mu=p^2(1-f)$, and

$$x=\frac{\mu}{2(1-f)^2}\left[2(1-f)+\mu-\sqrt{4(1-f)\mu+\mu^2}\right]$$

For small values of μ this approximates very closely to $\frac{\mu}{1-f}$ provided f differs appreciably from unity. Thus if $\mu=10^{-6}$, $f=.99$, the value of x is only 2 per cent. below $\frac{\mu}{1-f}$, a correction entirely negligible in view of our slight knowledge of mutation-rates.

The correction is still smaller in the case of a partially inbred population, where y is smaller for a given value of x.

If we take the members of wild populations which are fairly outbred, for example, *Drosophila melanogaster, D. obscura* and *D. sub-obscura, Trifolium pratense, Lolium perenne* and so on, we find that on inbreeding very large numbers of autosomal recessive genes more or less injurious in their effects are disclosed. Data and references to former work are given by Gordon (1936). These genes are not of course a fair sample of the mutants which appear in the species. They do not include dominants or sex-linked genes, which are not at all or only partially protected from selection by their normal allelomorphs. They also do not include incompletely recessive genes,

which cause an appreciable loss of fitness in the heterozygous condition.

For consider a gene of frequency p in a random mating population, whose heterozygotes have a frequency $2p(1-p)$ and fitness f_1, the homozygotes a frequency p^2 and frequency f_2. The gene appears at a rate $2(1-p)\mu$ and is eliminated at a rate $2p(1-p)(1-f_1)+2p^2(1-f_2)$. When these rates are equal,

$$p=\frac{k_1+\mu-\sqrt{(k_1-\mu)^2+4k_2\mu}}{2k_1-2k_2} \quad \text{where } \begin{matrix} k_1=1-f_1 \\ k_2=1-f_2 \end{matrix}$$

If μ is small compared with k_1 and k_2 this approximates closely to $\frac{\mu}{k_1}$ or $\frac{\mu}{1-f_1}$. In other words, the frequency of homozygous mutants is almost the same as with a rare dominant, *i.e.* negligible. The fraction of the total loss of fitness μ which is due to the homozygote is $\frac{k_2\mu}{2k_1^2}$, which is negligible unless $(1-f_2)\mu$ is of the order of $(1-f_1)^2$. In this case the loss of fitness to the species lies between μ and 2μ.

It is also clear that incompletely sex-linked genes (Haldane, 1936) will behave like autosomals, and that sex-limitation, incomplete manifestation and other like complications will not affect our results.

Since different genes mutate independently, they will be distributed independently in the population, even when linked. Hence if F be the fitness of the standard type, which is necessarily greater than unity, we have

$$F=\prod(1-m)^{-1}$$

where the multiplication is taken over all loci; and for any autosomal locus, m is the sum of the mutation rates of recessive allelomorphs, and twice those of dominant allelomorphs. For a sex-linked locus m is $\frac{3}{2}$ times the sum of all mutation rates.

$$\begin{aligned}\log_e F &= -\Sigma\log_e(1-m) \\ &= \Sigma m+\tfrac{1}{2}\Sigma m^2+\tfrac{1}{3}\Sigma m^3+\cdots\end{aligned}$$

But since every value of m is small, we have very approximately

$$\log_e F = \Sigma m$$

In other words the Malthusian parameter of the normal genotype is equal to the sum of the mutation rates of all deleterious genes and aberrations, multiplied by the factors 2 and $\frac{3}{2}$ in certain cases. If Σm is small, F approximates to $1 + \Sigma m$ and the loss of fitness due to mutation is Σm.

We must next consider other equilibria. Haldane (1927) showed that if the mutation rates of A to a and a to A are μ and ν, respectively, if $1-k$ be the fitness of aa when those of AA and Aa are unity, and if p be the frequency of the gene a in a random mating population, then if μ, ν and k (or $1-f$) are all small,

$$kp^3 - kp^2 - (\mu + \nu)p + \mu = 0$$

This equation has one or three real positive roots between 0 and 1. In the former case there is one stable equilibrium, in the latter two stable and one unstable. Here the loss of fitness to the whole population is kp^2, or $\mu - \frac{\nu p}{1-p}$ This is less than μ, the value where k is large compared with μ and ν, but is of the same order, and always positive. Thus if $\mu = .00007$, $\nu = .00018$, $k = .00016$, then $p = \frac{1}{4}, \frac{1}{16}$ of the population are recessives, and the loss of fitness is .00001, whereas if ν were zero, $\frac{7}{16}$ of the population would be recessives, and the loss of fitness .00007 or equal to μ.

Next consider the case where the heterozygote Aa is fitter than either homozygote AA or aa. Let the fitnesses of the three types be in the ratios $\alpha\, AA : 1\, Aa : \beta\, aa$. Let the actual fitnesses be $(1-k)AA$, $(1+h)Aa$, $(1-l)aa$. And let the frequency of the gene a be p, that of A q. We can neglect mutation, provided $1-\alpha$ and $1-\beta$ are much larger than the mutation rates. We find from the conditions of equilibrium

$$p = \frac{h+k}{2h+k+l} \qquad q = \frac{h+l}{2h+k+l}$$

From the condition that the mean fitness should be unity,

$$p^2l + q^2k = 2hpq,$$

Whence

$$h^2 = kl.$$

Also

$$\alpha = \frac{1-k}{1+h},\ \beta = \frac{1-l}{1+h}$$

Whence

$$h = \frac{(1-\alpha)(1-\beta)}{1-\alpha\beta},\ k = \frac{(1-\alpha)^2}{1-\alpha\beta},\ l = \frac{(1-\beta)^2}{1-\alpha\beta}$$

The total loss of fitness to the species is $(h+k)q^2 + (h+l)p^2 = h$, as is otherwise obvious. Hence, for example, if $\alpha = .99$, $\beta = .9$, that is to say if the two homozygous forms are 1 per cent. and 10 per cent. less fit than the heterozygous form, the loss of fitness is 0.92 per cent. for the species as a whole. In general $\frac{(1-\alpha)(1-\beta)}{1-\alpha\beta}$ is of the order of magnitude of the loss of fitness which would be caused if the whole species consisted of the fitter type of homozygote.

We see then that a single pair of genes causing increased fitness in the heterozygote has a far greater effect in lowering the fitness of the species than any gene which causes unfitness of a more serious character, provided that the heterozygote is not fitter than either homozygote. Hence there will be very strong selective influence in favor of any method by which the whole species may approximate to the phenotype of *Aa*. This may occur in at least four ways. By a mechanism of the *Oenothera* type the species may be kept in permanent heterozygosis. An allelomorph may appear which has intermediate effects between *A* and *a*, and which thus approximates to the phenotype of *Aa* when homozygous. If *A* and *a* are antimorphic a duplication may give a homozygote with *AA* in one pair of loci, and *aa* in another. Finally other genes may modify *AA* or *aa* towards the phenotype of *Aa*. In each of the last three cases the species will again become approximately homozygous.

In the type of equilibrium first discussed the Fisher effect will arise. That is to say, dominants will tend to become recessive, and recessives to disappear, owing to the presence of modifiers. If the spread of the modifiers is so slow that the species may always be considered in equilibrium for the main gene, it follows that the intensity of selection as measured by the loss of fitness will be unchanged throughout this process.

It remains to give some sort of estimate of the loss of fitness caused by mutation in a species. In *Drosophila melanogaster* the mean rate of mutation of sex-linked lethals per chromosome per generation is about .003, of autosomal lethals in the second chromosome .004, according to Muller (1928). According to Timofeeff-Ressovsky (1935) we may expect genes with no visible effect, but lowering fitness, to be about twice as frequent. The third chromosome probably behaves like the second. The total loss of fitness to the species is thus about $3(.003 \times \frac{3}{2} + .004 \times 2)$ or about 4 per cent. This may be taken as a rough estimate of the price which the species pays for the variability which is probably a prerequisite for evolution. The mutation rates of two human genes seem to be rather higher than those found in *Drosophila* (Haldane 1935, Penrose and Gunther 1935) if measured per generation, though not per year, so the figure for man is probably of the same order, though a little higher. In other words, if we could achieve the aim of negative eugenics and abolish all genes (including autosomal recessives, most of which can not even be detected at present) which seriously lower fitness in our present environments, we might expect a gain in fitness of the order of 10 per cent., though this might lower our capacity for evolution in a changed environment.

SUMMARY

In a species in equilibrium variation is mainly due to two causes. Some deleterious genes are being weeded

out by selection at the same rate as they are produced by mutation. Others are preserved because the heterozygous form is fitter than either homozygote. In the former case the loss of fitness in the species is roughly equal to the sum of all mutation rates and is probably of the order of 5 per cent. It is suggested that this loss of fitness is the price paid by a species for its capacity for further evolution.

REFERENCES

Fisher, R. A.
1930. "The Genetical Theory of Natural Selection." Oxford.
1935. *Jour. Genet.*, 30: 369–382.
Gordon, C.
1936. *Jour. Genet.*, 33: 25–60.
Gunther, M., and Penrose, L. S.
1935. *Jour. Genet.*, 31: 413–30.
Haldane, J. B. S.
1924. *Trans. Camb. Phil. Soc.*, 23: 19–41.
1926. *Proc. Camb. Phil. Soc.*, 23: 607–15.
1927. *Proc. Camb. Phil. Soc.*, 23: 838–44.
1931. *Proc. Camb. Phil. Soc.*, 27: 137–142.
1935. *Jour. Genet.*, 31: 317–326
1936. *Ann. Eugen.*, 7: 28–57.
Muller, H. J.
1928. *Genetics*, 13: 280–357.
Norton, H. P. J.
1928. *Proc. Lond. Math. Soc.*, 28: 1–45.
Sax, K.
1926. *Bot. Gaz.*, 82: 223–7.
Timofeeff-Ressowsky, N. W.
1935. *Gött. Nachricht. Math.-Phys. Klasse*, N. F., 1: 163–80.
Wright, S.
1931. *Genetics*, 16: 97–159.

ON THE EVOLUTION OF PHOTOSYNTHESIS[1]

DR. HAROLD F. BLUM
UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL

THE tremendous importance of photosynthesis for the development and maintenance of life upon the earth can hardly be overemphasized. In whatever manner life may have arisen, had it not found a means of building compounds of high potential energy by the addition of energy from the sun, its quantitative increase must have been narrowly limited, and it certainly could not have gone far on the road of evolution toward qualitative complexity. Biological chemosynthesis, which may be defined as the building of such high potential energy compounds by means of coupled reactions in which the over-all energy exchange results in a loss of chemical potential, could not have served because the total available potential energy must have been limited. The comparative rarity and simplicity of the organisms found in nature which maintain themselves by chemosynthetic activity testify to the very definite restrictions placed upon this type of life by the insufficiency of the chemical energy available for their maintenance and development.

The advent of photosynthesis must thus have been an extremely important step in the evolutionary process; but it is one which seems to be taken for granted by the majority of animal biologists, and even by plant biologists to whom the problem is somewhat more immediate although in a broad sense no more important. The principal purpose of this essay is to call attention to the uniqueness of the process of photosynthesis which is found in the higher plants, which we will refer to as "chlorophyll photosynthesis" to distinguish it from other types which we will discuss later. We will also attempt to show that the nature of this uniqueness makes it highly improbable that chlorophyll photosynthesis

[1] From the Division of Physiology.

could have been developed in a single evolutionary step, but that there must have been at least several distinct steps before the mechanism on which we are so vitally dependent for our existence was reached. A tentative scheme for the evolution of chlorophyll photosynthesis will be presented which may or may not be entitled to serious consideration, it is suggested only as a type of solution for the problem; but the seriousness of the problem itself in its relation to evolution as a whole can not be escaped.

Photosynthesis must have appeared quite early in evolutionary history, certainly before the Paleozoic era, when life forms had reached a state of considerable complexity, and ancestors of existing plant forms had already appeared which we may assume to have been equipped with the power of chlorophyll photosynthesis. If we grant the latter assumption, we are forced to the conclusion that this type of photosynthesis was in existence some time before the beginning of the Paleozoic era; and if, as we will attempt to show, it is too complicated to have arisen in less than several evolutionary steps we may expect the intermediate photosynthetic organisms to form a series extending back into and possibly beyond Archeozoic time.

There can be little doubt that the process of chlorophyll photosynthesis is a rather complicated one in comparison to other photochemical reactions and that it probably depends upon a high "organization" in the plant. It is questionable if the reaction has ever been successfully produced *in vitro* (see below), and it has at least one characteristic which is strikingly different from *in vitro* photochemical reactions in general. The chemical reaction involved in photosynthesis may be written as follows:

$$CO_2 + H_2O = CH_2O + O_2;\ \Delta F = +118{,}000 \text{ cals};\ \Delta H = +134{,}000 \text{ cals.}$$

If we wish, this may be considered only as a type reaction, CH_2O being regarded as an elementary carbohydrate building stone rather than as a molecule of formaldehyde. We may, however, calculate the free energy ΔF and heat

of reaction ΔH for the above reaction, assuming formaldehyde actually to be formed; the values are $\Delta F = +118{,}000$ cals./mol., $\Delta H = +134{,}000$ cals./mol.[2] We see that, since the reaction has a high positive free energy, it can not possibly go spontaneously in the direction indicated. In fact, it is highly important that the photosynthetic reaction is one in which energy must be put in, in order that we may later use this stored energy in the metabolism of the plant and animal. ΔF tells us whether the reaction will go spontaneously or not. ΔH tells us in this case how much energy we must actually put in to make the reaction go.

Now we know from modern photochemical theory that we can only put light energy into the molecule, and thus into a photochemical reaction, one quantum at a time. We may calculate the amount of energy in the quantum from the formula:

$$e = hc/\lambda$$

where e is the energy in the quantum, h is Planck's constant (6.56×10^{-27} ergs. secs.), c is the velocity of light (3×10^{10} cm./sec.), and λ the wave-length in cms. Now we know from the absorption spectrum of chlorophyll, and from experiment, that photosynthesis is brought about by light of wave-lengths up to about 7000 Å, approximately the long wave-length limit of human vision. This figure may thus be taken as that for the longest wave-length, and hence the smallest quantum which produces photosynthesis. The energy of such a quantum calculated from the above formula is about 2.8×10^{-12} ergs. To compare this value with that for the heat of reaction which is calculated in calories per mol. we must multiply it by the number of molecules in one mol. (6.06×10^{23})

[2] The data used in these calculations are those available in the International Critical Tables (1926), and Parks and Huffman (1932). The values for ΔF and ΔH should be of the proper order, and significant for our purposes, but can not be considered as exact since the concentrations of products and reactants in the plant are different than those for which the above data are determined. The more carefully calculated values of Stern (1933) are somewhat lower but of the same order and significance for our purpose.

and by the proper factor to convert ergs to calories ($.239 \times 10^{-7}$). The value which we obtain is about 40,000 calories per mol. If we compare this with the heat of reaction per mol. for the above reaction (134,000 calories per mol.) we see that one quantum of light per molecule would only be about one third that required, hence we must assume that at least three, probably four, quanta must be put into the photosynthetic process to reduce one molecule of CO_2 to formaldehyde. If we calculate the value for glucose or sucrose instead of formaldehyde we get approximately the same value—three or four quanta per molecule of carbon dioxide reduced; and this should be about the correct value for any carbohydrate that might be formed. We find experimentally (Warburg and Negelein, 1922) that under optimal conditions four quanta are used in the reduction of one molecule of CO_2 by the green alga *Chlorella*. But we see from the energy calculations that the process could not possibly use a less number of quanta of this size than three or four; and herein lies the uniqueness of the photosynthesis reaction when compared to other photochemical reactions, for we find, among the reactions which can be brought about by light *in vitro,* none in which the amount of energy which has to be added is greater than that contained in a single quantum of the light which brings it about. We find reactions in which many quanta are absorbed by the system in bringing about the reaction of a single molecule, but this is due to the fact that all the molecules which absorb light quanta do not react chemically. In the case of photosynthesis, it would seem that in some way four quanta of light must be captured and their energy added to the reacting molecules. It is probable that four steps are required, each taking one quantum of energy, but we need not be concerned here with the intimate mechanism of this reaction further than to say that it must be a much more complex one than is involved in any of those photochemical reactions which we definitely know to occur *in vitro*. Thus, it becomes difficult to find a way in which

this complicated mechanism could have originated in a single evolutionary step.

Riddle (1936) dismisses the question of the origin of photosynthesis with the assumption that formaldehyde, sugars and other organic compounds were first formed outside of living organisms, from H_2O and CO_2 by means of sunlight in the presence of colored surfaces. He bases this assumption on the experiments of Baly which he assumes as definitely proving the possibility of the above synthesis. Let us examine this concept more carefully. Baly, Heilbron and Barker (1921) and Baly *et al.* (1927a) claimed the formation of formaldehyde from CO_2 and H_2O by the action of ultraviolet radiation. If the energy for the formation of a formaldehyde molecule is to be supplied by a single quantum, this quantum must be about 9.4×10^{-12} ergs, which corresponds to a wave-length of about 2100 Å. Baly and his co-workers found it necessary to employ very short wave-lengths, as would be expected from the absorption spectra of the reacting substances, and we must assume that this reaction could only be forwarded by short wave-lengths of the order of 2000 Å. Actually, there is little or no radiation of this order in sunlight and we can not expect that there was appreciably more when photosynthesis originated, so that it is difficult to believe that very much carbohydrate originated in this simple direct way. With regard to the application of such a mechanism to the origin of the photosynthetic process of living organisms, we must recall that wave-lengths of this order are very destructive to organisms, and that chlorophyll photosynthesis is inhibited by them (Arnold, 1933). Hence a situation involving organisms very different than we know to-day must have existed if photosynthesis originated directly from such a reaction, and even so the jump from this process to the four quantum reaction characteristic of chlorophyll photosynthesis would remain difficult of explanation.

Subsequent experiments of Baly, Stephen and Hood (1927b) offer a somewhat more plausible explanation of

the origin of carbohydrate. They claim to have accomplished the synthesis of formaldehyde and carbohydrate by means of visible light when proper colored surfaces are present. It is assumed by Baly and Davies (1927c; and see also Baly, 1930) that these surfaces are "activated" and provide the necessary extra energy in addition to that which a single quantum of visible light may supply. This is possible thermodynamically, but in order for the first photosynthesis to have occurred in this way the "activated" surface must have been previously formed and supplied with energy; this would add to the number of events which must have occurred in proper sequence, and hence decrease the probability of such a spontaneous occurrence.

Even if we may assume such an origin for carbohydrates outside of living organisms, and possibly prior to the debut of life on earth, the problem of the origin of photosynthesis is not yet answered. For such a mechanism *in vivo,* the organism must provide the energy for "activating" the surface, and this energy must ultimately come from the sun if we assume a purely photosynthetic organism such as the modern higher plant. Thus the plant must still receive its three or four quanta for every molecule of CO_2 reduced to carbohydrate, so that the process of photosynthesis is not essentially simplified, nor is the origin of chlorophyll photosynthesis in a single evolutionary step explained. More recently, Baly (1935) suggests that two quanta of different wavelengths, one in the short and one in the long end of the visible spectrum, are received by two separate molecules, a chlorophyll A complex and a chlorophyll B complex, respectively; in this way about enough energy would be provided for the reduction of one molecule of CO_2 to carbohydrate. This would simplify our problem somewhat but not sufficiently.

It is only fair to state that other workers have not been able to confirm the experiments of Baly and his collaborators. Spoehr (1923), Porter and Ramsperger (1925),

Emerson (1929), Zscheile (1932) and Mackinney (1932) were all unable to obtain formaldehyde or carbohydrate synthesis *in vitro* by repeating various procedures of Baly and his group. As a result, Baly's findings have not met with general acceptance by plant physiologists and chemists, and the consensus of opinion seems to be that photosynthesis comparable with that in plants has not yet been accomplished *in vitro*. Reference to the paper of Dhar (1935) in the Third Cold Spring Harbor Symposia volume, and the accompanying discussion, should serve to convince the reader of the lack of agreement on this subject.

If we could give unqualified acceptance to Riddle's idea, the successive evolutionary steps filling the gap between the first photosynthesis outside the living body and modern chlorophyll photosynthesis would remain difficult. The writer will present an alternative scheme which provides a logical sequence of synthetic types leading up to modern photosynthesis. This would picture the first living systems on earth as chemosynthetic[3] rather than photosynthetic, that is, forms which actually used chemical potential energy in the synthesis of carbohydrates or other materials to be used in the structure of the organism or as fuel; this will be referred to as type A in the following discussion. In the earliest form of photosynthesis (type B) the light quantum may have served only to supply energy of activation and thus to speed up a reaction which could go spontaneously without the addition of energy, as is the case for many *in vitro* photochemical reactions. Later, organisms may have evolved in which carbohydrate or other organic material was formed by some reaction in which only one quantum was necessary to provide the energy to forward a reaction which would not go spontaneously (type C). From these systems, organisms might have evolved which used two or three quanta (type D) until chlorophyll photosynthesis requiring four quanta (type E) was finally evolved.

[3] Lipman (1924) has made the suggestion on other grounds that chemosynthetic organisms appeared earlier than photosynthetic.

Such a scheme must be necessarily speculative, but no more so than much of the accepted reasoning about the evolutionary problem. Some evidence might be provided if we were able to show the existence of organisms which might represent the intermediate steps which we have indicated above, thus following a well-accepted method often employed in the morphological approach to the problem of evolution. Chemosynthesis in living organisms (type A) was first discovered by Winogradsky in 1886 and at present a considerable number of such forms are known to exist, while numerous others are suspected. Baas-Becking and Parks (1927) have reviewed the literature on the subject, and have shown that none of these organisms on which enough data exist to make energy calculations possible disobey the second law of thermodynamics, if the synthetic process may be regarded as a coupled reaction in which carbohydrate is formed and in which there is an over-all loss of free energy. Type B is not essential for the scheme, and as yet we know of no example of this. Types C and D may perhaps be represented by the green and purple sulfur bacteria which van Niel (1931) has proved to be photosynthetic through a series of beautiful studies. Van Niel has provided the data from which chemical reactions may be assigned to the photosynthetic processes of these organisms which are able to synthesize bacterial substance when grown in inorganic media provided CO_2, H_2S and light are present; they do not produce O_2 in the process. Thus the chemical reactions are distinctly different from that of chlorophyll photosynthesis, and, moreover, the light-absorbing pigment is not chlorophyll.

Van Niel (1931) gives the following reaction for the green bacteria, which is based on careful quantitative studies of the stoichiometric relationships of products and reactants:

$$CO_2 + 2\,H_2S = CH_2O + H_2O + 2\,S;\ \Delta F = +18{,}000 \text{ cals.};\ \Delta H = +22{,}000 \text{ cals.}$$

It will be seen by examining the free energy values that this reaction could not go spontaneously, so that it can not

belong to type B. On the other hand, we see that only one quantum of light of 7000 Å (40,000 cals.) would be required in the case of these organisms to provide the necessary energy ΔH. Thus we might assume that the green sulfur bacteria are representative of type C.

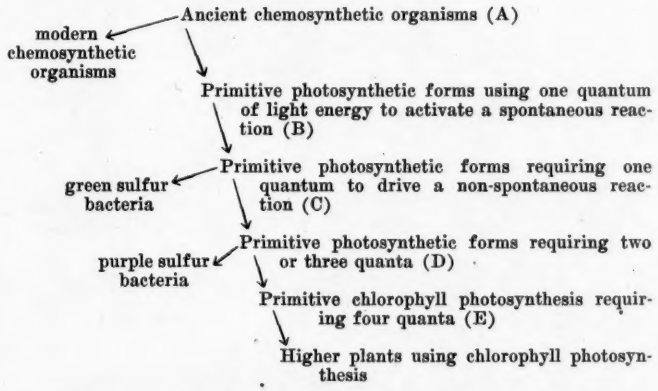
The purple sulfur bacteria (*Thiorodaceae*) have an over-all reaction as follows (van Niel, 1931):

$$2\ CO_2 + H_2S + 2\ H_2O = 2\ CH_2O + H_2SO_4;$$
$$\Delta F = 66{,}000 \text{ cals.}; \quad \Delta H = +77{,}000 \text{ cals.}$$

This reaction can not go spontaneously and would require two quanta of red light, or one quantum per molecule of CO_2 reduced to carbohydrate. The studies of van Niel (1931) indicate that the first step in this reaction is the oxidation of H_2S to S, according to the equation for the green bacteria, since the purple bacteria are able to oxidize S and also sulfite and thiosulfate to sulfate. They may also produce S when grown in the presence of H_2S. This would indicate at least a two-step reaction so that we might place these bacteria as representatives of type D. It would seem possible that organisms of the type of the green bacteria were at first unable to carry the oxidation of H_2S farther than S, but later developed the ability to add a further step by using another quantum to oxidize S with the reduction of another molecule of CO_2. Measurements by Roelofsen (1935) indicate that four quanta or more are required by the purple sulfur bacteria for the reduction of one molecule of carbon dioxide, and van Niel (1935) uses this as evidence that the photosynthesis in these forms is of the same essential type as that of chlorophyll photosynthesis, but it must be pointed out that this is not necessarily an indication of such a similarity, for it may simply mean that for every quantum used in chemical reaction three quanta are absorbed and the energy in some way dissipated. This is a common occurrence in photochemical reactions *in vitro*.

However, from the standpoint of our hypothesis it is sufficient to show that photosynthetic forms exist in which the reduction of CO_2 *could* take place with the use of only

one and two quanta. We would not wish to say that the existing green and purple bacteria may be regarded as actual intermediate forms but only as demonstrating actual photochemical processes which might have furnished the necessary steps in the evolutionary chain leading to four quanta chlorophyll photosynthesis. According to a hypothesis of the writer presented in an earlier paper (1935), it is highly improbable that the ancestral forms of any of the existing organisms are present on the earth to-day—the forms existing to-day should not, therefore, be considered as intermediate but as the evolutionary descendants of intermediate forms. We might present the following scheme which is similar to one which we introduced on an earlier occasion (Blum, 1935).



In this scheme it is assumed that all the ancestral forms of life have disappeared, only modified descendants being left. Thus, if the existing purple sulfur bacteria actually do follow a process of four one-quantum jumps, as suggested by van Niel, this may be a late development; and perhaps we should place these bacteria on a more direct line toward the chlorophyll-bearing plants, which would call for a slight modification of the above scheme. The fact remains that forms using the reaction of the green sulfur bacteria, and using only one quantum of light, and forms using the reaction of the purple sulfur bacteria and

two quanta may have existed and furnished the necessary steps for evolution to the four quantum chlorophyll photosynthesis.

It is of interest to note that the purple sulfur bacteria are facultative in that they are able to use somewhat different chemical reactions according to the environment (see van Niel, 1935). They may use in their photosynthetic process organic substances as well as the sulfur compounds mentioned above (van Niel, 1935). Such a facultative ability would be important from an evolutionary standpoint because it indicates that various photosynthetic mechanisms have been developed by the organism and thus presented for natural selection. This is further emphasized by the existence of the *Athiorodaceae*, a group of photosynthetic purple bacteria which do not require sulfur compounds but use organic substances in the absence of O_2 when light is present. We have made no attempt to place the *Athiorodaceae* in our evolutionary scheme, since we do not know their chemical reactions nor the energies required; but they serve as further evidence that photosynthesis has evolved in more than one direction.

As van Niel (1931, 1935) points out, all the photosynthetic reactions which have been demonstrated may be represented by a scheme in which CO_2 is reduced by a hydrogen donor. Thus H_2O is the hydrogen donor in chlorophyll photosynthesis, and H_2S in the green sulfur bacteria. In the purple sulfur bacteria the hydrogen donor may be H_2S; a further oxidized sulfur compound; or, as also in the case of the *Athiorodaceae,* an organic compound. The evolution of photosynthesis might, from this evidence, be conceived to have proceeded through the use of a series of hydrogen donors; the selection of each donor being dependent upon the energy requirements of the particular photosynthetic reaction in which it might take part.

Our scheme must remain a tentative one, and we can not take our hypothesis of intermediate steps too seriously

for the present. However, as stated above, we must regard the evolution of photosynthesis as an important basic problem in the consideration of the total evolutionary process. The suggestion may be made that the study of the chemosynthetic and primitive photosynthetic organisms should be an extremely rich one for those interested in the problem of those early evolutionary stages which must have so profoundly influenced the direction of later evolution. It is probable that many organisms of this group and their chemical reactions remain undiscovered, and it is only by the isolation of such forms and careful chemical studies that we can hope to obtain the necessary information for a thorough consideration of this problem. The question would seem closed from a paleontological point of view because we can hardly hope that any records of the primitive ancestral forms are left. They may be lost somewhere in the Archaeozoic or even in the Azoic rocks. Green algae are reported from the Archeozoic, but if these were truly chlorophyll-bearing forms they represent a considerable evolutionary development and the events which interest us here must lie behind them. The purpose of this essay will have been fulfilled if the magnitude of the problem of the evolution of photosynthesis has been called to the attention of biologists, and if it may help to point out the necessity of a consideration of energetics as a fundamental approach to the study of evolution.

LITERATURE CITED

Arnold, W.
1933. *Jour. Gen. Physiol.*, 17: 135.
Baas-Becking, L. G. M., and G. S. Parks
1927. *Physiol. Rev.*, 7: 85.
Baly, E. C. C.
1930. *Nature*, 126: 666.
1935. *Proc. Roy. Soc.*, 117B: 218–239.
Baly, E. C. C., J. B. Davies, M. R. Johnson and H. Shanassy
1927a. *Proc. Roy. Soc.* (London), A116: 197.
Baly, E. C. C., W. E. Stephen and N. R. Hood
1927b. *Proc. Roy. Soc.* (London), A116: 212.
Baly, E. C. C., and J. B. Davies
1927c. *Proc. Roy. Soc.* (London), A116: 219.

Baly, E. C. C., I. M. Heilbron and W. F. Barker
1921. *Jour. Chem. Soc.* (London), 119: 1025.
Blum, H. F.
1935. AMER. NAT., 69: 354.
Dhar, N. R.
1935. *Cold Spring Harbor Symposia on Quantitative Biology*, 3: 151.
Emerson, R.
1929. *Jour. Gen. Physiol.*, 13: 163.
International Critical Tables
1926. New York, McGraw-Hill.
Lipman, C. B.
1924. *Scientific Monthly*, 19: 357.
Mackinney, G.
1932. *Jour. Am. Chem. Soc.*, 54: 1688.
van Niel, C. B.
1931. *Arch. Mikrobiologie*, 3: 1.
1935. *Cold Spring Harbor Symposia on Quantitative Biology*, 3: 138.
Parks, G. S., and H. M. Huffman
1932. "The Free Energies of some Organic Compounds." Chem. Cat. Co., New York.
Porter, C. W., and H. C. Ramsperger
1925. *Jour. Am. Chem. Soc.*, 47: 79.
Riddle, O.
1936. *Science*, 83: 41.
Roelofsen, P. A.
1935. "On Photosynthesis of the *Thiorhodaceae*." Thesis, Utrecht.
Spoehr, H. A.
1923. *Jour. Am. Chem. Soc.*, 45: 1184.
Stern, K.
1933. "Pflanzenthermodynamik." Julius Springer, Berlin.
Warburg, O., and E. Negelein
1923. *Ztschr. phys. chem.*, 106: 191.
Zscheile, P. F.
1932. *Jour. Am. Chem. Soc.*, 54: 973.

SARGASSO WEED FISH "NESTS"

MADE BY FLYING FISHES NOT BY SARGASSO FISHES (ANTENNARIIDS): A HISTORICAL SURVEY

E. W. GUDGER

BIBLIOGRAPHER AND ASSOCIATE CURATOR OF ICHTHYOLOGY, AMERICAN MUSEUM OF NATURAL HISTORY

I HAVE long had it in mind to write the history of the discovery of the curious bands of jelly in which are embedded the eggs of the sargasso fishes and of the "nests" which these bands are supposed to help form. A letter recently received from a valued English ichthyological correspondent asking for data on these egg-bands has crystallized that intention.

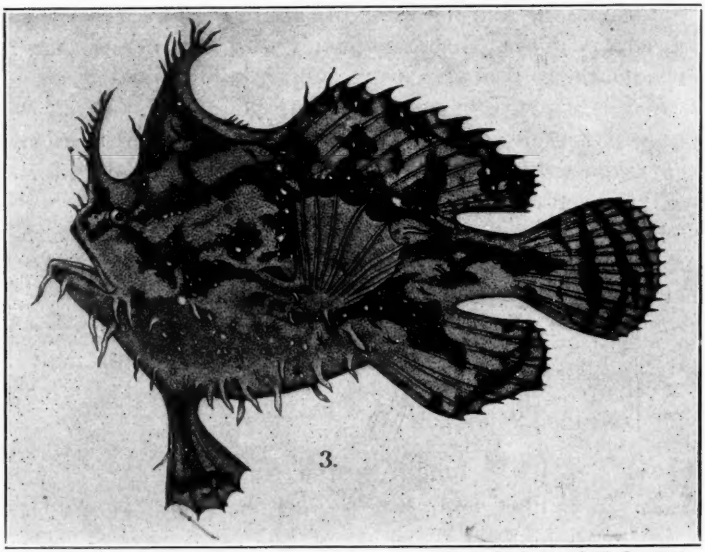
The sargasso fishes are so named because they live in clumps of sargasso weed, holding on to the fronds by means of their semi-palmate pectoral fins. While climbing about in the *Sargassum,* these fishes have been supposed to extrude their bands of egg-containing jelly, which are presumed to bind these clumps of weed together into so-called "nests."

THE SPAWNING SEEN

In July, 1903, while working at the Beaufort, N. C., Marine Biological Laboratory of the U. S. Bureau of Fisheries, I had brought to me two living specimens of *Pterophryne histrio* (Fig. 1). The larger of these, being fed bountifully, showed a progressively enlarging abdomen, and presently laid a long egg-raft which floated at the surface of the water. This raft of non-adhesive clear gelatinous material with embedded eggs in volume amounted to about 250 cc. The raft had greatly swelled in the water, since the fish had a volume of only about 80 cc. After oviposition, the fish's abdomen was reduced to normal size and shape. The egg-raft and eggs plainly accounted for the "fatness" of the fish. In my description (1905) I unfortunately used the word "string" instead of

the exact term band or raft. There was no "string." The eggs, about 1 mm in diameter, were thickly embedded in the band of jelly. The eggs were of course unfertilized, and development stages unfortunately could not be followed. The phenomenon was reported in 1905.

In June, 1913, while at the Tortugas Laboratory of the Carnegie Institution of Washington, I collected a number



After Jordan and Sindo, 1902.

FIG. 1. The sargasso fish or marbled angler, *Pterophryne histrio*, the alleged "nest" builder.

of specimens of *Pterophryne gibba* from *Sargassum* blown in from the Gulf Stream. These were kept in aquaria with gulf-weed and some of them laid egg-rafts practically identical with those studied in Beaufort. It was noted at the time that each egg-raft presented a slightly pebbled appearance, due to the myriads of small embedded eggs. I had to go to Key West the next day and could not study the egg-raft further. Other layings were made by the fish during my enforced absence, but on

my return the rafts had gone to pieces. This phenomenon I recorded in 1929.

It was noted at Tortugas that, when Pterophrynes were put in aquaria without gulf-weed, they were very restless and unhappy, but when *Sargassum* was supplied they climbed into it and their restlessness ceased. It must then be explicitly understood that the egg-raft layer was in an aquarium with gulf-weed, that the egg-raft floated at the surface of the water free of the weed, and that the fronds of the *Sargassum* were not bound together in a ball by the egg-band.

The publication of my note in 1905 brought me a letter from Dr. H. M. Smith, of the U. S. Bureau of Fisheries, telling me that in 1897 great numbers of *P. histrio* had been brought into Woods Hole by a succession of southerly winds. Many were kept in his aquaria, and several of these spawned. The eggs were embedded in long bands like those of the goosefish (*Lophius*). Dr. Smith made incidental record of this phenomenon in 1898, but unfortunately his article had never been brought to my attention. His record in 1898 of this phenomenon is the first ever made.

According to Gill (1907), Smith again witnessed this phenomenon in September–October, 1906, when three sargasso fish spawned in his aquaria at Woods Hole. These rafts were transparent, non-adherent, and floated easily. They were from 450 to 920 mm long, 75 mm wide and about 6 mm thick. The eggs were 0.6 mm in diameter, very numerous, "thickly infiltrating the jelly." Concerning all these egg-rafts, Smith wrote me (October 24, 1935) that "The gelatinous matter in the rafts was not tenacious, elastic or inclined to form threads." And my later observations (in 1913) absolutely confirm Smith's. Smith fortunately photographed a portion of one egg-band, as may be seen in Fig. 2 herein. This is the only known figure of the raft.

The only other observer of this egg-laying known to me is Hornell (1921), superintendent of the Madras, India,

Aquarium. He records this habit in *Antennarius hispidus* as follows: "They occasionally spawn in the tanks; the eggs, extremely numerous and tiny, are embedded in a colourless gelatinous band-shaped sheet, relatively of enormous size compared with the parent. One such sheet, deposited early in September, 1919, measured 9½ feet in length with a width of 6¼ inches."

When in 1905 I rediscovered the egg-raft of a pelagic angler fish, I was too new in zoology and too ignorant of these fishes to know that similar egg-bands are laid by the littoral angler fishes, and that a similar habit might be predicated of the sargasso fishes. During the summer of 1871, Baird had seen what the fishermen of New England call "a purple veil," a mucous band 20 or 30 feet long and 4 or 5 wide with embedded purple specks about 30 to the square inch. A bit of this was submitted to Alexander Agassiz, who identified it as coming from the goosefish (*Lophius*). But unfortunately, Agassiz did not publish on the subject until 1885.

However, in 1880, Captain Collins of his own knowledge made known the structure of the "purple veil" and its origin in the ovaries of *Lophius*. Had I known of Collins's note (1880), my own account (1905) of the egg-laying of *Pterophryne* would have been linked with the same habit in the other angler fishes.

"Nests" Described but Oviposition Not Seen

The foregoing accounts are of egg-raft laying in aquaria. Presumably none of the writers ever saw a "nest." There will now be briefly noted and discussed the accounts of a number of men who saw the "nests" but did not see the egg-raft extruded.

The first of these is Louis Agassiz, who sent from St. Thomas, December, 1871, a description (published in January, 1872) of a pelagic fish egg-nest. Agassiz's article, the first description ever published of this curious object, excited wide-spread interest. It was reprinted at home and abroad in at least five different journals. Fur-

After Gill, 1909.

FIG. 2. Egg raft with embedded eggs of *Pterophryne*, from a photograph by H. M. Smith.

thermore, as such things generally do, it loosed a small flood of similar observations. For all these reasons, it seems well to copy here the significant parts of Agassiz's account of the nest of *Chironectes pictus,* as he called it.

> It was a round mass of sargassum about the size of two fists, rolled up together. The whole consisted, to all appearance, of nothing but Gulf weed, the branches and leaves of which were, however, evidently knit together, and not merely balled into a roundish mass; for, though some of the leaves and branches hung loose from the rest, it became at once visible that the bulk of the ball was held together by threads trending in every direction, among the sea-weed, as if a couple of handfuls of branches of sargassum had been rolled up together with elastic threads trending in every direction. Put back into a large bowl of water, it became apparent that this mass of sea-weed was a nest, the central part of which was more closely bound up together in the form of a ball, with several loose branches extending in various directions, by which the whole was kept floating.
>
> A more careful examination very soon revealed the fact that the elastic threads which held the Gulf weed together were beaded at intervals, sometimes two or three beads being close together, or a bunch of them hanging from the same cluster of threads, or they were, more rarely, scattered at a greater distance one from the other. Nowhere was there much regularity observable in the distribution of the beads, and they were found scattered throughout the whole ball of sea-weeds pretty uniformly. The beads themselves were about the size of an ordinary pin's head. We had, no doubt, a nest before us, of the most curious kind: full of eggs too; the eggs scattered throughout the mass of the nest and not placed together in a cavity of the whole structure.

In the light of the three descriptions of the egg-rafts above, let us critically examine this account of the nest. Agassiz describes "branches and leaves [of *Sargassum*] . . . knit together . . . by elastic threads trending in every direction." Also he says that "the elastic threads . . . were beaded at intervals, sometimes two or three being close together, or a bunch . . . hanging from the same cluster of threads, or . . . scattered at a greater distance . . ." Unfortunately Agassiz did not present a figure of his "nest."

Whatever Agassiz had before him, he did not have the egg-bands of a sargasso fish. By no sort of chance could the *bands,* described by Smith, Hornell and myself, have been transformed into "elastic threads." They are gelatinous, but not sticky and elastic; they are bands, not strings or threads.

Agassiz did not see the nest in the making, nor did he find a *Chironectes pictus* in it. However, he hatched some of the embryos but could not carry them far enough to determine the genus and species. But by comparing their chromatophores with those of a young captive *C. pictus* he found them identical, and on this finding he declared the nest to be that of this fish.

This seems to me to be a rather far-fetched method of identification, and I seriously doubt its correctness. If the identification is accepted as correct, it can only be on a basis of the conjecture of Gill (1907, 1909) that some sargasso fish eggs had got mixed with another kind of eggs in this "nest." Sargasso fishes clambering around in a mass of gulf weed might at the same time have oviposited egg-bands and thus some of their eggs might have gotten mixed with the other kind.

This extensively republished paper from so distinguished a scientist aroused wide-spread interest, which resulted in numerous descriptions from various hands. These will be noted in the briefest possible fashion. It may be stated once for all that none of these "nests" contained egg-*bands*.

J. M. Jones (1872) describes from Bermuda a nest "woven together by a maze of fine elastic threads . . . from which depends the clustering mass of eggs. . . These threads are amazingly strong, especially at their terminal bases, . . . are apparently twisted together like the fibres of a rope . . . thereby rendering the fabric . . . solid and secure."

Hinde (1872) collected in the Sargasso Sea ". . . a mass of weed compactly woven by strong, white, silky fibres into a round ball . . . [whose] surface . . . was covered with a network of these fibres to which large numbers of glassy eggs were attached . . . their cases were very tough."

Goode (1876) obtained *P. picta* at Bermuda and saw ". . . its curious nest, consisting of bunches of eggs adhering in glutinous masses to the *Sargassum*, the whole cluster large enough to fill a quart measure."

From the "Voyage of the Challenger" come two accounts of nests. Thompson (1878) figures an *Antennarius marmoratus* found among sargasso weed and writes of ". . . very strong transparent strings of the viscid secretion of this fish . . . and of spaces among the fronds filled with eggs." Moseley (1879) speaks of a nest of weed bound together by "long sticky gelatinous strings."

S. G. Jones (1884) collected in the Sargasso Sea and kept in aquaria aboard his ship marbled anglers and nests with eggs. In the sea he had noted adults, always by twos, "excitedly playing" around nests and evidently "examining them." When he put an adult in a jar with a nest it at once "darted into the middle of the nest and I saw it no more." Then he adds, "I have often seen *small* Anglers on the inside, but I think that they remain there for protection until they are big enough to take care of themselves on the outside." It seems to me that these phenomena are merely cases of seeking protection in the masses of seaweed, and not necessarily for places to spawn.

Vaillant (1887) describes in very indefinite fashion nests taken on the cruise of the *Talisman*. The fronds of the *Sargassum* are held together by "liens . . . plus forts." These are apparently made up of "filaments d'une extrême ténuité (0.010 à 0.015 mm)." These filaments are of uniform caliber save for enlargements where they are attached to the eggs. These elements are united in more or less large numbers to form the "liens," which have a diameter of 0.5 mm.

Ives (1889) found a nest in the collections of the Philadelphia Academy and says of the fish that "It makes its peculiar nest by binding together the fronds of the seaweed with gelatinous threads, and depositing the eggs throughout the mass."

Martens (1872) translates Agassiz (1872) and discusses nest-building in general. Geare (1903) merely abstracts Agassiz. Presumably neither man ever saw

the fish in the water and probably neither ever handled a nest. References to them are added for completeness.

These are all the modern accounts of alleged sargasso fish "nests" known to me. But for the reader's attention there are now to be set forth two accounts of this so-called nest which have long lain hidden from ichthyologists. And being apparently the earliest known suggestions, they seem worthy of quotation in a historical paper.

Peter Osbeck, a pupil of the renowned Linnaeus, went as chaplain on a Swedish East India ship bound to China and return. The journal of his voyage was published in his native Swedish at Stockholm in 1757. A German translation was issued at Rostock in 1765, and an English version from London in 1771. My quotation is from this last.

On May 22, 1752, the ship being in the "Grass-sea," Osbeck brought on board, studied and described some sargasso weed (his *Fucus natans*). In this description we read as follows:

> . . . the globose parts of fructification were (like some of the leaves, stalks, and branches) harder than usual; occasioned, as it seemed, by the slime which sometimes fastens itself on the leaves, branches, or other parts: in this some very small blackish grains, or rather eggs of crabs, and insects, are inclosed: when these insects, afterwards forsake their habitations, they leave marks in the hardened slime behind them. Sometimes a slime exceedingly like the whites of eggs sticks to the leaves, in which an innumerable quantity of snail's eggs joined together make a white or yellow chain, like a *Taenia*, so wound backwards and forwards that one can neither find its beginning or its end.

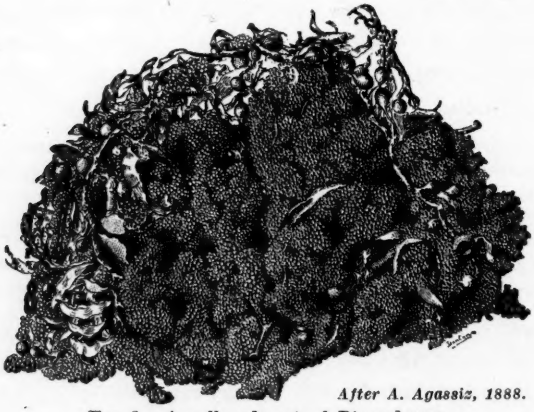
Osbeck concludes as follows: "If this . . . is not Dampier's fish-roe which is said to swim in the Sargazo, I have not met with it." The index to Masefield's edition of Dampier's "Voyages" (London, 1906) giving no help, I went through the volumes page by page and at last found what Osbeck presumably had in mind. Dampier's "Voyage to New-Holland . . . in . . . 1699" was published in London, 1729. In Masefield's edition of the "Voyages," in volume II, page 420, Dampier writes: ". . . the sea was full of a sort of small Grass or Moss, which as it floated

in the Water seem'd to have some Spawn of Fish: and there was among it some small Fry."

This observation was made slightly east of the center of the Indian Ocean, on a traverse from the Cape of Good Hope to Shark's Bay, West Australia. What this intrepid adventurer and keen observer of natural history actually saw I can not say. His account is included for what it is worth. It seems that Osbeck probably had a "nest" like those previously described.

FIGURES OF THE "NESTS"

There are only three representations of the alleged nest of an antennariid fish. The first of these we owe to Alexander Agassiz (1888). Here is his representation—my Fig. 3. All that he says of it is "Pterophryne, . . . of

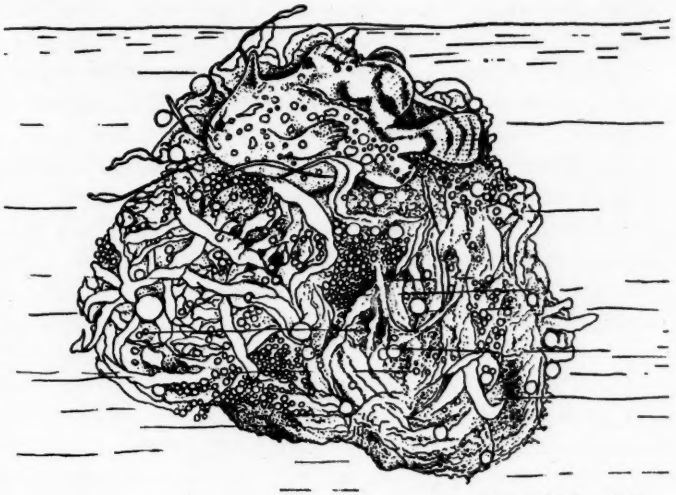


After A. Agassiz, 1888.

FIG. 3. An alleged nest of *Pterophryne*.

the Sargasso Sea, is specially adapted to live among the floating algae, . . . , in which it twines its gelatinous clusters of eggs."

The next known figure, my No. 4, is from the facile pen of H. W. Fowler (1928). Nothing is said about it in the text, but Fowler kindly writes: "The drawing was made from a specimen fish with its 'nest' and was copied as carefully from nature as I was able. As I recall, it was



After Fowler, 1928.

FIG. 4. *Pterophryne* on its "nest."

obtained somewhere in the Gulf Stream off our Atlantic coast, where these so-called nests are not rare."

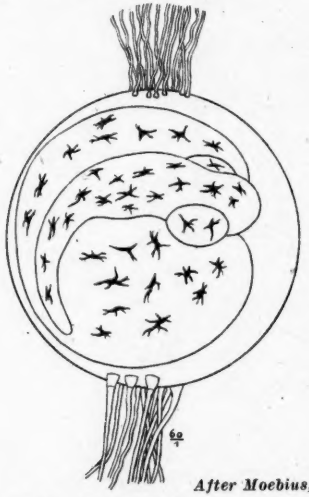
We are now come to the consideration of the figure and description of a "nest" which at first complicated the problem but which eventually led to its being cleared up.

SARGASSO FISH "NESTS" MADE BY FLYING FISHES

In 1894, Moebius figured and described a dried "nest" from the equatorial Atlantic as the product of a sargasso fish. His figure is reproduced on soft paper and shows no details whatever. Hence it does not seem worth while to reproduce it. In his description he states that the gulf-weed was held together by strings, the thicker of which were 1 to 3 mm in diameter. The strings in turn were composed of numerous very fine threads to which eggs were suspended. By treating the nest with suitable re-agents, Moebius was able to see and remove such eggs as he portrayed—my Fig. 5. These were 1.67 mm in diam-eter, and from their poles originated numerous threads having enlarged bases of attachment. These were of two

sizes, the larger being jointed at their base. Some of these threads were 12 mm long. They varied in diameter from 8 to 24 microns. The threads of the nest-strings to which the eggs were suspended were 8 to 16 microns in diameter.

Moebius examined another and similar nest—from the Gulf of Guinea. Its composition was very similar to that of the preceding. The strings binding the nest together had hanging from them eggs with numerous bipolar filaments similar to those seen in Fig. 5.



After Moebius, 1894.

FIG. 5. Egg of a flying fish, mistakenly attributed to *Pterophryne* (×30, not 60).

Moebius inspected the ovarian eggs of *Pterophryne* and found no filaments, but conjectured that the eggs might be provided with these as they passed down the oviduct. However, both Smith (1898) and Gudger (1905, 1929) have found that *Pterophryne* eggs embedded in the raft have no filaments. Moebius is too cautious to allege that these nests were made by sargasso fish, but thought this "highly probable."

Gill, in 1905, after examination of the egg-rafts of *Pterophryne* submitted to him by Smith and by the writer,

decided that these egg-bands had no part in the making of such a nest as that described by Moebius. Then he asked—"Can such be the product of a flying fish?"

In 1907 and again in 1909, Gill answered his own question in the affirmative. Studying Moebius's paper, he recognized the eggs with bipolar filaments taken from the nest as those of a flying fish. This led to the conjecture that the "nests" above described were all made by the eggs of the flying fish. "The eggs must be laid on the fronds of the weed and the long motile tendril-like filaments clasp the finely cut branches of a frond till a globular mass is brought together." To test this hypothesis, Gill obtained from A. Agassiz some eggs from the nest shown in my Fig. 3, and—found them to be the eggs of a flying fish. Here then it was demonstrated that in materials and mode of formation the nest figured by A. Agassiz was identical with that figured and described by Moebius.

Corroborative of Gill, I have, through the courtesy of H. W. Fowler, of the Academy of Natural Sciences of Philadelphia, been able to examine a bit of the "nest" described by Ives (1889). In this I find a mass of white tough elastic fibers securely tying the eggs to the fronds of sargassum. Carefully teasing some of the eggs out of the mass, I have been able to see the clusters of bipolar filaments growing out of the egg-shells. Here then there is to me ocular proof that the "nests" in the sargasso weed are composed of the threads and attached eggs of a flying fish and not of the egg-raft of the sargasso fish which I first saw in 1903. These tendril-like filaments lay hold of the fronds and, aided by the rolling action of the waves, round the *Sargassum* up into globular masses.

Now let the reader go back through the accounts set out, and note that attention is constantly called to "elastic threads," some of these "beaded with eggs"; to "threads . . . twisted together like the fibers of a rope"; to "a network of these fibers"; to "strong transparent strings"; to "filaments d'une extrême ténuité."

These descriptions fit the egg-strings of the flying fishes figured and described by Moebius, seen by Gill in A. Agassiz's material and by me in the nest described by Ives, but do not fit the egg-bands of the Antennariids, since neither Smith (1898) nor Gudger (1905, 1929) found any polar filaments attached to the shells of the eggs of their *Pterophryne* egg-rafts.

Finally some additional present-day evidence can be brought forward, from a far distant part of the world. Hornell (1923) went out from the Coromandel coast with native fishermen seeking flying fish. Bundles of twigs with leaves attached to floats at the extremities of long coir ropes were cast out and allowed to float barely submerged. Around these lures the flying fish would collect and when the lines were slowly and carefully pulled in the accompanying fish were captured with dip nets. Hornell found that the flying fish collected around the leafy bundles not for shade and shelter but as a suitable substratum on which to deposit their spawn.

On examining the bundles, Hornell found that:

> The branches and leaves of the shrub were full of a tangled-up multitude of tiny colourless eggs with innumerable glassy threads, tough and elastic, attaching them in masses to one another and also to the leaves and branches of the plant. . . . The filaments attached to the eggs are sometimes surprisingly long and highly elastic, and admirably adapted to tangle the eggs securely among the leaflets of floating seaweed (Sargasso-weed chiefly) which is undoubtedly the natural object for the purpose.

Unfortunately Hornell's figure is too small and too indistinct to show eggs and threads intertwined among the leaves and branches, hence it is not reproduced. However, Nayudu (1923) figures and describes these eggs with their filaments. They are very like the eggs figured by Moebius, and those that Gill found from Agassiz's nest and that I find in the bit of nest sent me by Fowler.

What Hornell's photographs fail to show, we find in Beebe's figures published nine years later—1932. Beebe had long known of Agassiz's reputed discovery, and in the course of his work on the fishes of Bermuda he had constantly been on the watch for these alleged nests. He

found five nests. "The first three I accepted with no more misgiving than a medieval monk would doubt the

After Beebe, 1932.

FIG. 6. A sargasso weed fish-nest collected at Bermuda in 1931. The small white bodies are the eggs, the larger ones are the air-floats of the sargassum weed.

The weed was tightly packed into a ball about four by five inches in diameter, and was held together by many very strong turns of white string. When cut these contracted at once into a tangle of numerous fine, silken threads. The eggs were in three general stages of development, and when I unwound several feet I found that there were three separate masses of these stages, hinting strongly that three female fish had taken part in the laying. The most important thing was that each egg had a number of very thin hairs attached to it, a character peculiar to a family of fish which includes flying fish.

dictum of Aristotle." In the fourth the eggs were dead, but in the fifth (collected in 1931) all were alive. This nest is shown in Fig. 6, and here follows Beebe's definite description of it.

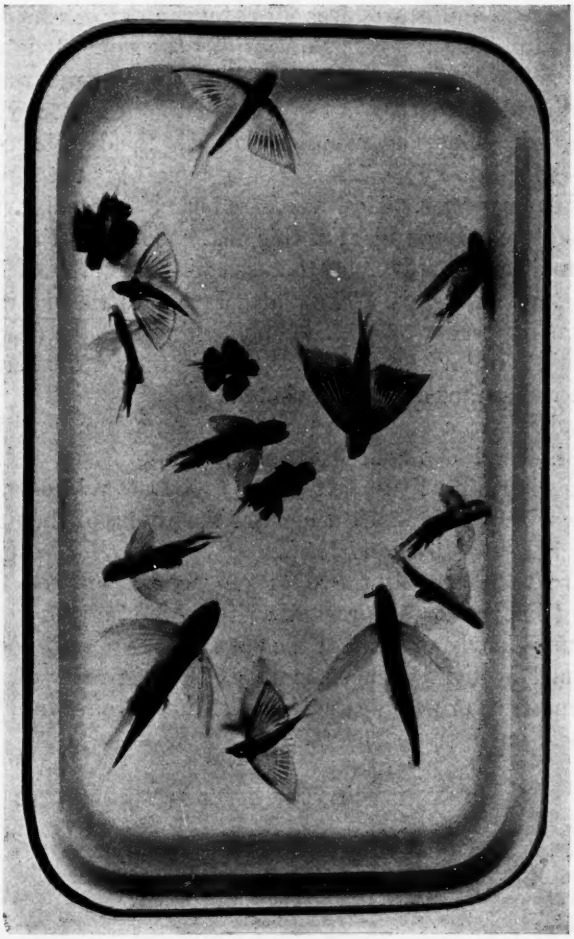
What is more to our purpose, Beebe made a much enlarged photograph of a part of this nest. This is repro-

After Beebe, 1932.

FIG. 7. A portion of the sargasso weed fish-nest much enlarged. Note the eggs with their threads—especially one in the lower left field. The large rounded objects are the air-floats attached to the sargassum stems.

duced herein as Fig. 7. In this are seen many eggs and a number of these have the threads shown very clearly. These eggs were thought surely to be those of a flying fish, and this was proved beyond question when these ova were carried on to hatching and the young reared to the stage where their "wings" enabled Beebe to assign them to the genus *Exoñautes*.

Thus 60 years after the publication of Agassiz's erroneous conclusions (1872), the question of the sargasso weed fish nest was settled by Beebe finally and for all time, and



After Breder, 1934.

FIG. 8. A pan of young flying fishes. The three little fellows with solidly black fins are the young of the "four-winged" *Exonautes rondeleti*.

by the most convincing of all methods—hatching, rearing and photographing.

These results were confirmed by Breder while on a cruise in the West Indies in 1934. He took a number of

the "nests" and reproduces a photograph of one held in the hand in the air. It has essentially the same structure as the nest figured by Beebe (No. 6 herein). Breder also reproduced in another figure a bit of the nest somewhat magnified. This, however, does not give the detail shown in Beebe's figure (No. 7 herein) and hence it is not reproduced in my paper. However, Breder makes an observation that no other student of this nest has noted—that the eggs and cords are heavier than sea water and that if left alone they sink in it. Their attachment to the buoyant sargassum weed keeps them from sinking into the deeper and colder water and maintains them in the warm surface water where the eggs quickly hatch.

Breder kept a part of a nest in an aquarium until the eggs hatched out and grew into young flying fishes. These charming little fishes are to be seen in Fig. 8. Those with solidly black wings are the young of the nest-builder, *Exonautes rondeletii.*

Here then are all the data on "sargasso-weed fish nests" known to the present writer. A study of these accounts clearly shows that the eggs in these "nests" are spawned by flying fishes, and that the egg-rafts of the Antennariids or pelagic angler fishes do not enter into the composition of these "nests."

LITERATURE CITED

Agassiz, Alexander
1888. "Three Cruises of the . . . 'Blake'." (Nest of *Pterophryne*, II, p. 311.)

Agassiz, Louis
1872. *Amer. Jour. Sci. and Arts*, 3. ser. 3: 154–155.

Baird, S. F.
1871. AM. NAT., 5: 785–786.

Beebe, William
1932. "Nonsuch: Land of Water." (Sargasso weed nest and eggs, pp. 68–72, pls. 16 and 17).

Breder, C. M., Jr.
1934. *Bull. N. Y. Zool. Soc.*, 37 (Flying-fish nests, pp. 32, 37 and 41); 2 figs.).

Collins, J. W.
1880. *Forest and Stream*, 15: 8.

Fowler, Henry W.
1928. *Mem. Bishop Mus.*, Honolulu 10 (Nest of *Pterophryne*, fig. 82).
Geare, R. I.
1903. *Sci. Amer.*, 89: 218.
Gill, Theodore
1905. *Science*, n. s. 22: 841.
1907. *Science*, n. s. 35: 63–64.
1909. "Angler Fishes: their Kinds and Way." Smithsonian Rept. for 1908 ("Spawning and Nests of *Pterophryne*," pp. 607–615, 5 figs.).
Goode, G. Browne
1876. *Smithsonian Misc. Colls.*, 13 (*Pterophryne picta*, p. 20).
Gudger, E. W.
1905. *Science*, n. s. 22: 842–843.
1929. *Carnegie Institution of Washington, Pub.* No. 391 (*Pterophryne gibba*, p. 203).
Hinde, George G.
1872. *Nature*, 6: 142.
Hornell, James
1921. Guide to the Madras Aquarium.
1923. *Madras Fish. Bull.*, 15: 99–108, 2 pls.
Ives, J. E.
1889. *Proc. Acad. Nat. Sci. Phila.*, 41: 344–345.
Jones, J. Matthew
1872. *Nature*, 5: 462.
Jones, S. Griffith
1884. *Proc. Lit. and Phil. Soc. Liverpool*, 38: liii–lvi.
Martens, Eduard van
1872. *Zool. Garten*, 13: 107–116.
Moebius, K.
1894. *S. B. K. Preuss. Akad. Wiss.*, Berlin (pt. 1), 1203–1210, 3 figs.
Moseley, H. N.
1879. "Notes by a Naturalist on the *Challenger*." (Nest of *Antennarius*, p. 567).
Nayudu, M. Ramaswami
1923. *Madras Fish. Bull.*, 15: 109–112, 3 figs.
Osbeck, Peter
1771. "A Voyage to China and the East Indies." London. Trans. by John Reinhold Forster. (Sargasso weed nest?, II, pp. 111–112.)
Smith, H. M.
1898. *Bull. U. S. Fish. Comm. for 1897*, 17 (*Pterophryne histrio*, p. 109).
Thompson, C. Wyville
1878. "The Voyage of the *Challenger*." (*Antennarius marmoratus*, I, p. 188; II, p. 17.)
Vaillant, Léon
1887. *C. R. Mém. Soc. Biol.*, Paris, 8. ser. 4: 732–733.

THE HYDROID POLYP CORYMORPHA PALMA AS GESTALT AND AS HISTORY

DR. FRANCIS G. GILCHRIST

Introduction

A fundamental problem of biology concerns the genesis of the organism: Is the organism an achievement, the product of a history lived; or is it a realization, the result of an equilibrium attained? Is development a straightforward, creative process, from the germ cell or bud onward? Or is it a regulation toward the specific individual pattern which a given sort of living matter always seeks to assume? In a word, is protoplasm to be compared to a historic something, such as a skyrocket, which bursts into an ever-expanding pattern and then fades away? Or is it to be compared to a Gestalt or configuration, such as a candle-flame, which continually tends to assume and to preserve its characteristic form and structure? In the one case, time means something to the organism, for development gets somewhere. In the other case, time means little; and development may be backward (as in rejuvenation) as well as forward.

As embryologists we have been inclined to the first of these two alternatives. We have noted the determinations and differentiations by which the egg becomes progressively structurized into a mosaic of regions having unlike capacities in development. We have witnessed that irreversible drift of form and function which is the life cycle. As students of regeneration, on the other hand, we have tended toward the second alternative. We have been impressed with the remarkable regulative powers of the organism, especially of lower organisms, and by their capacity to attain the same specific pattern by more than one route. This dilemma between the historic and the Gestalt view-point is fundamental in biology; as it is also in psychology and the social sciences.

With this problem in mind, let us turn to one of the simpler organisms and inquire of it experimentally as to whether it is an achievement or a realization, a history or a configuration, a skyrocket or a candle-flame.[1]

Material

Corymorpha palma (Fig. 1) is a large solitary hydroid frequently found in abundance on the mud of tide flats in southern California.[2] Each polyp consists of a hydranth (head or "hydroid flower"), a naked stem and a base to anchor it in the mud. The hydranth is composed of several zones: At the distal end is the mouth, subtended by (a) a zone bearing short, agile distal tentacles. Beneath this is (b) an intertentacular zone, hollowed out internally to form a gastric cavity or stomach. Then comes (c) a zone bearing a single whorl of long, slender proximal tentacles; then (e) a sub-tentacular zone; and finally (f) a constriction or neck. Below the hydranth is the long, tapering naked stem, which in large polyps may make up three fourths or more of the length of the animal. The center of the stem is occupied by large pith cells of entodermal origin. Several entodermal ducts run between the pith and the ectoderm of the outer body wall, and open, at their upper ends, into the gastric cavity. The conical base of the polyp secretes about itself a delicate, transparent sheath of perisarc; and from its middle third, it gives origin to several longitudinal double rows of rootlet buds

[1] The work here reported was begun in the department of zoology of Pomona College, and was aided by grants from Claremont Colleges and the American Association for the Advancement of Science. It was continued while the author was a guest of the Scripps Institution of Oceanography, La Jolla, California. To this institution and to its staff he is grateful for the many courtesies extended.

[2] Regeneration in *Corymorpha palma* has been previously described by Torrey (1910 a and b), and by Child (1926 a, b and c; 1927 a and b). The process is very similar to that of the related colonial hydroid Tubularia (studied by Loeb, Bickford, Driesch, Peebles, Morgan, Child and others), differing in that the hydranths of Tubularia are short-lived and are promptly cast off when injured. Moreover, the stem of Tubularia is almost completely covered with perisarc, and the lower portion of each polyp is a branching stolon.

(probably reduced stolons). These grow out one by one, beginning with the lowest of each row, and penetrate downward into the mud. As they grow, the living tissue of each bud (the coenosarc) loses connection with that of the polyp proper, uses itself up and in a short while per-

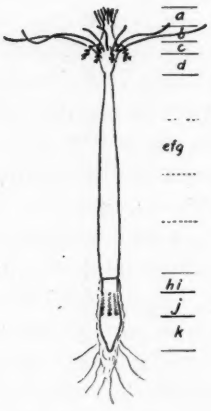


FIG. 1

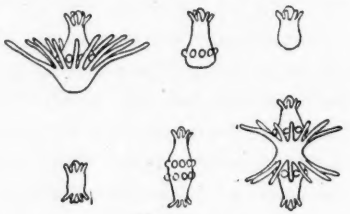


FIG. 3

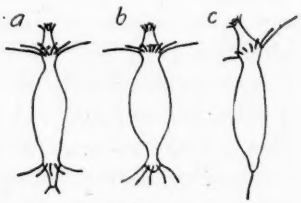


FIG. 4

FIG. 1. Corymorpha palma. Entire polyp showing zones and levels of cutting. The lettering refers to the regenerates illustrated in Fig. 2.

FIG. 3. Primary incompleteness, both monapical and biapical, as seen in regenerates of very short pieces of the naked stem. Redrawn from Child (1926c).

FIG. 4. Secondary incompleteness, as shown in regenerates of the naked stem. a. Lower hydranth with two distal tentacles only; b, with no distal tentacles; and c, with one proximal tentacle in terminal position.

ishes; but the perisarc which it secretes as it grows outward remains as a holdfast filament to anchor the polyp in the mud.

EXPERIMENTS

Our first experiment, designed to discover whether Corymorpha is an achievement or a realization, will be to cut the polyp transversely into pieces, and then observe

to what extent the fragments thus isolated possess the power of remodelling themselves into wholes. If they remain as differentiated parts, we shall conclude that this organism is an achievement, the product of a true history past, a flower in fact, which injured in the bud must ever show the injury until it perishes. If, however, the pieces regulate themselves into new wholes, we shall decide that the polyp is a realization, a system which tends toward an equilibrium, a true Gestalt.

Let us then subdivide the polyp as indicated in Fig. 1. The several pieces thus separated are each (except the stem pieces) from a distinctly different part of the animal. Three to six days later we examine the fragments and find that all have reconstituted themselves; but to different degrees and in different ways. The result may be described as follows:

(1) The fragments of the hydranth have failed to reform whole new polyps. (a) The zone of distal tentacles has either rounded off at its cut surface, or it has regenerated a second "heteromorphic" zone of distal tentacles and a second mouth (Fig. 2a). (Heteromorphosis is the regeneration of something else than the part removed.) (b) The intertentacular zone, if it lives, has regenerated a mouth and distal tentacles at the upper cut surface, and may have formed a second mouth, zone of distal tentacles and intertentacular zone at its lower cut surface (Fig. 2b). (c) The zone of proximal tentacles (including also the zone of gonophores) has not lived unless the gonophores and tentacles were entirely cut away. If this was done, it has formed a mouth, zone of distal tentacles and intertentacular zone at its upper cut surface (often it forms more than one mouth) and a second "heteromorphic" hydranth with proximal as well as distal tentacles from its lower cut surface. It may, however, have produced a perisarc-covered base from its lower surface; or both a hydranth and a base (Fig. 2c). The gonophores and proximal tentacles which were cut off have begun to be replaced. (d) The subtentacular zone and neck have

produced hydranths from both cut surfaces (Fig. 2d); or they have produced a hydranth from the upper surface and a base from the lower.

The general principle which stands out in these results is that a given zone of the hydranth has the capacity to

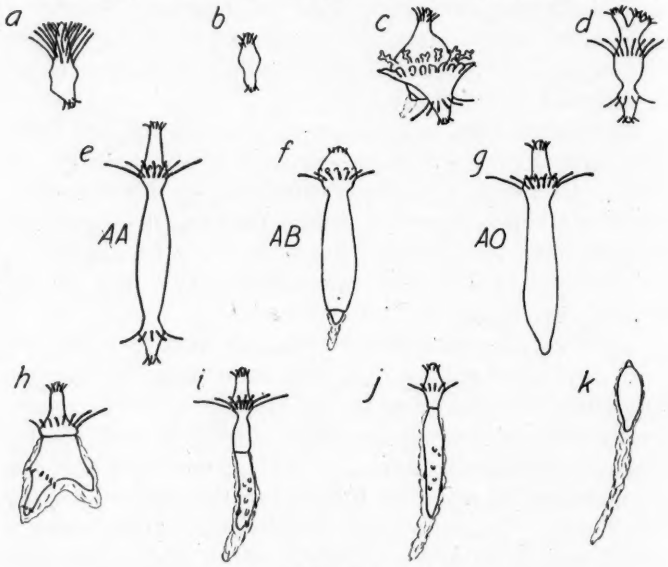


FIG. 2

FIG. 2. Regenerates from fragments of the hydranth, naked stem and perisarc-covered base. a. Zone of distal tentacles (with some of the subjacent zone). b. Intertentacular zone. c. Zones of gonophores and proximal tentacles (with portions of adjacent zones); note the development from the lower surface of both a hydranth and a base. d. Subtentacular zone and neck (with a little of the upper end of the stem); note the multapical upper end. e, f and g. Biapical (AA), apicobasal (AB) and monapical (AO) regenerates from pieces of the naked stem. The upper hydranths illustrate three phases in the rhythmic extension and contraction of identical hydranths. h. Upper zone of base (with some of the naked stem); note tendency to multipolar regeneration. i, j and k. The three zones of the base.

regenerate zones which are distal to itself, and to do this even when in doing so the polarity or direction of the parts is reversed. A given level at a cut surface does not regenerate any level of the hydranth proximal to itself, al-

though, strangely enough, some levels of a hydranth may form a base. We shall call this the *principle of proximo-distal regeneration;* and shall have more to say about it presently.

(2) The pieces of the naked stem do any one of several different things. Ultimately most of them form complete polyps with hydranth and base; but they do not always do so directly. Each piece forms a hydranth at its upper end; but at its lower end it may form either a hydranth, a perisarc-covered base, or nothing at all (Figs. 2e-g). In other words, the piece may become either (a) biapical (AA), with a hydranth at both ends; (b) apicobasal (AB), with a hydranth at the upper end and a base at the lower; or (c) monapical (AO), with a hydranth at the upper end and nothing at all at the lower.[3] Biapical regenerates usually form a base after a few days from some point between the hydranths (Torrey, 1910b); but especially if they are long, they may fail to do so. Monapical regenerates may persist as such for a long time.

Short pieces of stem may form either one or two hydranths of nearly the same size. Sometimes in very short pieces the hydranth or hydranths are incomplete (Fig. 3). It is as though the hydranth were planned on a scale larger than the size of the piece. When this occurs it is always the distal zones which are produced; more or less of the proximal portions which are lacking. We shall term this condition *primary incompleteness.* It was originally described in the hydroid Tubularia by Bickford, Driesch and Morgan; and has been studied in Corymorpha by Child (1926c). Longer pieces also frequently form two hydranths, but in this case the lower hydranth is usually smaller than the upper. It may also be incomplete (Fig. 4). In this case it is the distal zones, not the proximal, which are most reduced in size or are lacking. In the extreme example figured, the regenerate consists of a full-

[3] Child (1927 a, p. 347) prefers a different terminology. He groups apicobasal and monapical forms as unipolar. Biapical forms he calls bipolar, except when they form bases from the side of the stem. In this case he terms them bipolar-unipolar.

sized, complete hydranth at the upper end, but of only single tentacles at the tip of the lower end.[4] We shall call this *secondary incompleteness.* The phenomenon has not been discussed, although it is figured by Child (1932, Fig. 14; 1935, Figs. 11–13) in Corymorpha, and a comparable condition by Weimer (1928, Fig. 18) in a regenerate of Hydra.

These several types of regenerates of the stem are most significant, and have been studied extensively and statistically by Child (1926c, 1927), who, among other things, finds that the proportions of biapical regenerates is greatest in pieces of about one fourth the length of the naked stem, and in pieces taken from the middle or just above the middle of this portion of the polyp.

Two principles stand out clearly in the different types of stem regenerates which have just been described: (a) the principle of *polarity,* according to which the two ends of a piece of stem tend to regenerate differently; and (b) the principle of *variability of the lower end;* that is, the lower end may do any one of several different things.

(3) Fragments of the perisarc-covered base have the power of regenerating whole polyps. A segment from the upper end of the base usually regenerates rapidly in an apicobasal fashion, with a hydranth at the upper end and the zones necessary to complete the base at the lower end (Fig. 2i). Not infrequently, however, such a piece forms a heteromorphic hydranth at the lower end (biapical regeneration, Fig. 2h), which soon becomes encased in the perisarc secreted above it and dies. Thus the polarity of regeneration has been reversed, while the polarity or direction of perisarc production has not. This illustrates the relative independence of hydranth-forming and base-forming processes. The zone of rootlet buds and even the

[4] Proximal tentacles are readily distinguished from distal tentacles by their long, slender, tapering form; their dilated tip, which is not opaque; and their comparative inactivity when not stimulated. Distal tentacles, on the contrary, are shorter, of nearly uniform diameter throughout, are knobby by reason of numerous nematocysts, are opaque at the tip and are agile and continually in motion.

terminal zone below the rootlet buds may regenerate whole polyps; but the hydranths which are produced are usually small and slow in their rate of development (Fig. 2j and k).

In general we note two principles in the regeneration of fragments of the Corymorpha polyp: (a) the principle that *no part of the polyp is without some power of regeneration;* and (b) the principle that *no two parts have the same power of regeneration.* The capacity for regeneration is greatest in the naked stem, especially just above the middle of the naked stem, and becomes less and less as the two ends of the polyp are approached.

What, then, is the answer which the Corymorpha polyp gives to the question as to whether it is a realization or an achievement; a Gestalt or a history? The answer is not categorical. Nature, in fact, rarely gives us a clear-cut answer to our most precisely formulated questions. The polyp is both a realization and an achievement. It is a realization and comparable to a candle-flame because a part may regulate itself and go at least some distance toward producing a whole. It is an achievement and to be compared to a skyrocket because no two parts produce a new whole in the same way, and because some parts fall short of producing a whole.

Analysis

Let us, however, pursue our problem further. How can an organism be at one and the same time an achievement, the product of a germ cell past; and the realization or attainment of an equilibrium which was to come? Can living matter look two ways at once? Can development be both centrifugal (from the germ outward) and centripetal (toward a specific pattern)? An adequate answer to this question will require a careful and somewhat detailed analysis of the processes of structurization by which the new polyp, in particular the new hydranth, is produced.

As a first assay at this analysis, let us suppose that a human workman were to undertake to form a hydranth

from a piece of material of cylindrical shape. He would no doubt begin at the end of the piece and measure out the portions to be assigned to each zone. He would then cease his measuring and commence the process of modeling his "hydroid flower." He would not begin with the proximal zones and work outward, for he would not know just where to begin, and moreover, when he was finished he might have material left over, or perchance, not sufficient material to complete the hydranth.

Does nature go about the process of forming a hydranth in this same fashion? Does she begin at the tip and measure inwards, zone by zone? What answer does experiment give?

(1) The phenomenon which we have called *primary incompleteness* (Fig. 3) clearly gives an affirmative answer to the question (*cf.* Child). In very small pieces of stem the hydranth is sometimes laid out on a scale too large for the size of the piece. Only a portion of the hydranth is then produced, and it is always the tip and distal portions which are formed. When material runs out, it is the proximal portions which are lacking. Nature most obviously begins her measuring at the tip.

(2) The phenomenon of proximo-distal regeneration (Fig. 2a-d) apparently gives a negative answer to our question. When a part of a hydranth is removed, the wound heals, a cone grows outward (whether by proliferation of new cells, migration of undifferentiated cells or redifferentiation of old cells need not concern us here), and differentiation takes place. Only those zones of the hydranth are formed which are distal to the cut surface. This is equally true whether the cut surface be the upper surface or the lower surface of the piece. It is as though the new part which is formed were an *extrapolation* from the old tissues of the cut surface outward. Now, this principle of proximo-distal regeneration is widely observed in the animal kingdom. It is as true of salamander's limbs and lizard's tails as it is of hydranths. It would seem that nature, in laying out the new portions of a terminal organ,

works from the older tissues outward, and adjusts the scale of her measurements to fit the amount of material available. (Przibram, 1924, 1931, has studied these phenomena very extensively).

Now, can it be possible that the structurization of a whole new hydranth from a piece of stem is from the tip inward; while the structurization of a completing portion of an injured hydranth is from the old tissues outward toward the tip? This is not reasonable. We are in need of a more careful analysis of the process.

The difficulty with our first analysis is not far to seek. We assumed that our hypothetical workman began by measuring off from the tip the portion of the material to be assigned to each zone. We omitted an important step. What he actually did was first to survey the piece and comprehend it as a whole. Only then was he in a position to choose the "tip" from which to begin his measurements (for a tip is a tip only in relation to the whole). He chose also the direction in which to measure. Furthermore, if he was a good workman and not in too much haste, he probably observed the size of the material at his disposal and adjusted the scale of measurement accordingly, in order that the hydranth when finished might be proportional to the whole.

Does nature go about her work in this same methodic fashion when she structurizes a hydranth or a salamander's limb or a lizard's tail or, for that matter, when she hews out a new organism from an egg or from a portion of an egg? Does she progress by these same three steps? A. First, does she comprehend the material as a whole, choose the place to begin, the direction in which to progress, and the scale? B. Does she then measure out the material to the parts and assign to each the rôle which it is to play? C. Lastly, when these first two steps have been accomplished, does she begin the processes of actually molding the material into its final form and structure? There is indeed abundant reason to believe that nature does proceed in this fashion and by these three

steps. But before we proceed with the evidence as it relates itself to the formation of a polyp, it will be well to restate our analysis of structurization in less pictorial and more physical terms.

The structurization of an organ; for example, of a hydranth, passes through three phases: A, a phase of *organization* in which a simple quantitative pattern or gradient-field is established within the region; B, a phase of *invisible differentiation,* in which qualitative differences arise within the region, as evidenced by the parts acquiring definite and diverse capacities (potencies) for development; and C, a phase of *visible differentiation,* in which the invisible potencies which have arisen express themselves in different developmental activities such as thickenings, thinnings, inpocketings, outpocketings, cell migrations and tissue differentiations.[5]

A. By organization a gradient or field originates which gives order and unity to the region undergoing structurization. In the case of the hydranth the tip of the regenerating piece becomes the high end of the gradient (Child) or center of the field (Weiss). If, as sometimes happens, two tips appear, then a double gradient results, and a double structure, for example, a hydranth with two mouths and two distal zones, is differentiated (see Fig. 2d). The field also establishes the polarity of the piece. (In higher organisms it establishes the bilaterality and asymmetry as well.) The steepness of the gradient determines the scale on which the differentiations occur, and

[5] The distinction here drawn between *organization* and *differentiation* is in part that of Weiss (1926 b); although this author includes most of what I have termed *invisible differentiation* under his concept of organization. His statement (1927) that differentiation is directly under genic control and hence capable of analysis by the methods of genetics is true of what I term *visible differentiation.* The term *gradient-field* is taken from Huxley and DeBeer (1934); and is, of course, an adaptation of the concept of physiological gradients, as held by Child, and that of the embryonic field, as advanced by Gurwitsch and Weiss. The term *invisible differentiation* has been used in preference to germinal localization, embryonic segregation, determination and chemo-differentiation.

hence the final size of the hydranth.[6] Within limits, the smaller pieces give rise to the smaller hydranths; although, as we have seen, in very small pieces, especially when from fresh, vigorous material, the scale of the hydranth may be larger than the piece (primary incompleteness). The gradient-field is also highly labile, for it readily adjusts itself to the form of the piece and to the forces of the surroundings. (Any differentiation already present in the piece will of course tend to stabilize and anchor the field.) Most important of all, the field has always the aspect of wholeness. A half of a zone of proximal tentacles will form a whole regeneration cone, which, when it differentiates, will produce a whole zone of oral tentacles. (Exactly the same principle applies to the regeneration of limbs of salamanders.) In short, the gradient-field is simple, quantitative and labile. It gives unity, direction and dimensions to the differentiations which follow. It has always the aspect of wholeness; and when it has been established, the parts within the field have definite prospective fates (provided of course that there is no later interference with the course of development). If it were not for this process of organization differentiation could not possibly give rise to an integrated organ, but would produce a hodge-podge or heap.

B. By *invisible differentiation* the zones of the hydranth are determined. We may picture the process as a wave of chemical change which, beginning at the tip (that is, at the center of the gradient-field) sweeps over the field, changing in its specific nature as it goes.[7] First a material

[6] The steeper the gradient, the farther the dominance of the high end of the gradient extends, and the larger is the scale upon which the hydranth is structurized. The gradient at the upper end of a piece is steeper than that at the lower end, and larger hydranths result. Depressing conditions lower the gradient, and produce smaller hydranths. Increased stimulation at the cut surfaces, such as results from placing the pieces in diluted sea water, increases the steepness of the gradient and results in larger hydranths. There is evidence, however, that the matter is not as simple as this, and further study of the problem is needed.

[7] This view of invisible differentiation as beginning at the center of the gradient-field and spreading outwardly, applies to organs of determinate differentiation; for example, to heads and limb buds. It does not apply to

is elaborated which imparts to the terminal zone the capacity to produce oral lips and distal tentacles. Then a zone is determined which forms no tentacles, but which hollows out to form a gastric cavity or stomach. Next a formative stuff is deposited which imparts to the region in which it lies the power to form gonophores. The next zone in turn develops the potency of forming proximal tentacles; and so on. Even the upper portion of the stem is somewhat determined hydranth-ward, as is evidenced by the fact that pieces from the upper portion form larger hydranths, and show a greater degree of polarity (more apicobasal regenerations) (Child, 1926c). In short, invisible differentiations are qualitative processes, which result in the acquisition by limited areas of powers or potencies for development which they did not before possess.[8] Invisible differentiations, finally, are partial processes, in the sense that, as they progress, the parts acquire more and more the power to self-differentiate as parts when isolated.

C. In due time the potential organ rudiments undergo *visible differentiation;* that is, they express themselves in diverse developmental activities; and the hydranth begins to take form.

Let us now attempt to apply these three phases of structurization to the phenomenon of proximo-distal regeneration. The cut surface of the piece of hydranth heals. A cone-shaped projection grows out. While this is going on, A, organization takes place, and a gradient-field is established in the cone, with its high end at the tip. The field adjusts itself at its lower end to the pattern of differ-

indeterminate differentiations, such as tails and plant stems. In the latter cases differentiation is frequently rhythmic, each successive process of differentiation beginning nearer the tip than the last one; but the tip itself remains undifferentiated. The result is metamerism.

[8] By invisible differentiation the *prospective organ rudiments*, the fates of which are a function of their position in the gradient-field, become *potential organ rudiments* (preprimordia) with inherent capacities to develop according to their nature.

entiation already present in the old tissues.[9] **B.** Invisible differentiation now begins at the tip and progresses in a *disto-proximal* (*sic*) direction, and continues until the zones which have been differentiated make *harmonious juncture* with the old differentiated tissues at the base. Differentiation is thus not an extrapolation from the old tissues outward, as the principle of proximo-distal regeneration superficially seems to indicate; rather it is an *interpolation* between the tip and the old tissues. A distal zone is always formed because differentiation begins at the distal end. The new part which regenerates is always a whole, because the field in which the differentiation occurs is a whole. **C.** After the preprimordia of the zones of the hydranth have thus been determined, visible differentiation begins. Sometimes the visible primordia of the distal tentacles appear first; more often the proximal tentacles are the first to be seen. The visible differentiations of the definitive primordia are thus independent of one another; and it does not follow that, because invisible differentiation was from the tip inward, the visible organ rudiments will appear in the same order.

(3) The principle of polarity (Fig. 2c-k), namely, the principle that the two ends of a regenerating piece of stem commonly behave differently, gives us some insight into the nature of the gradient-field. Are we to think of polarity as due to the orientation of the intimate structure (for example, the molecular structure) of the stem? If so, then the living material is comparable by way of analogy to magnets and crystals. Or are we to conceive of polarity as the expression of a gradation, analogous, for instance, to a rod which is continuously heated at one end and cooled at the other, so that between the two ends a gradient of potential and a flow of energy exists? The first concept

[9] In the regeneration of the salamander's limb, the gradient-field takes not only its polarity, but its entire three-dimensional orientation and laterality from the old tissues of the cut end of the stump (Milojević, 1924). Its unity, however, and its wholeness are independent of the stump, but are determined by the unitary dominance of the tip (Weiss, 1926a; Milojević and Vlatkovic, 1926).

was that of Loeb and Driesch and has been defended strenuously by Przibram. The second concept is the kernel of the hypothesis of physiological gradients of Child. The entire problem has been extensively discussed.

Let us attempt to analyze this matter experimentally. If the field be of the nature of a magnet, then the smallest piece will be just as much polarized as a large piece. If, on the other hand, the field is comparable to a rod heated at one end and cooled at the other, then the smaller the piece, the less will be the difference of potential within the piece, and the less will be the evidences of polarity. Now, in the flatworm Planaria, the smaller pieces actually do give an increased number of biapical regenerations; that is, decreased evidence of polarity (Morgan, 1904). In Corymorpha, however, the number of biapical regenerations increases down to pieces one fourth of the naked stem in length (Child, 1926c). Smaller pieces than that show more rather than less evidence of polarity (*i.e.*, increasing proportions of apicobasal regenerations). But, as Child points out, this is at least in part due to the smaller pieces standing on end more frequently than the larger pieces.[10] The evidence thus favors the gradient theory. Child (1926a and b) has given us independent evidence of various gradients in the stem of Corymorpha: respiratory, electrical, in susceptibility and in rate of penetration of dyes. More recently he and Watanabe (1935; Watanabe, 1935) have stained regenerating pieces with methylene blue, and have then watched the progress of the decolorization (reduction) of the methylene blue. In pieces destined to monapical regeneration, the reduction begins at one end (the upper); in pieces destined to biapical regeneration, the reduction begins at both ends.

It is, however, not unlikely that the field is both a gradation and an orientation; an orientation, that is, of dynamic

[10] Child shows that very short pieces of stem may stand on either their upper or their lower ends. In both cases the free end becomes a hydranth, while the end in contact with the substrate is apt to become a base. In this paper, however, we are concerned with the rôle of intrinsic factors rather than with differential forces of the environment.

forces rather than of intimate structure. Indeed, one may inquire whether a gradation could exist without some aspect of orientation. In this case the field is analogous to a configuration such as an electric or a magnet field (*cf.* Burr and Northrop, 1935), in which the forces are oriented with respect to the center of the field, and in which also they diminish in intensity with increasing distance from the center.[11] The entire problem is in need of exact observation and analysis.

Now, the initial field which gives polarity to regeneration is inherited by the piece from the parent stem, and has, no doubt, its material basis in some sort of invisible differentiation present in the stem.[12] But the field in relation to which differentiation actually takes place is this field after it has been profoundly modified by the cutting and the processes of healing which immediately follow. The fact that a hydranth may form at the lower as well as at the upper end of a piece is ample evidence that this is true. We can not, therefore, speak of hydranth-forming and base-forming poles as one speaks of the north-seeking and south-seeking poles of a magnet. The fact that different sorts of regeneration commonly occur at the lower end than take place at the upper end is not finally a problem of polarity at all, but one of determination. What happens is this: The stimuli at the cut surfaces, and more especially the increased metabolism of healing, alter the field. The gradient at the upper end is steepened; that at the lower end is antagonized and quite commonly reversed. Thus two fields are formed within the piece with

[11] The field which develops within the blastema of a regenerating amphibian limb possesses both orientation and gradation (Weiss, 1926b). Moreover, the orientation is three-dimensional, and the limb which develops is either a right limb or a left limb, depending upon the laterality of the stump. There would seem to be no adequate physical analogy for such a configuration, combining as it does gradation with orientation in three dimensions of space.

[12] Small pieces taken from the upper and lower ends of the naked stem show more evidence of polarity than pieces taken from the middle (Torrey, 1910b; Child, 1926c). This presumably is because the upper end is more differentiated hydranth-ward; and the lower end is more differentiated base-ward (compare Morgan, 1906).

their centers at the upper and lower ends, respectively. But why, if this is so, does the lower end not always form a hydranth?

(4) The principle of the *variability of the lower end* in regeneration (Fig. 2e-g), requires explanation. It will be recalled that in the regeneration of pieces of stem the upper ends invariably form hydranths; the lower ends may form hydranths, bases or nothing at all. What determines which of these structures shall form?

The key to the problem is to be found in experiments in which the upper end is prevented from interfering with the processes of regeneration at the lower end. For example, if the upper end be held firmly in contact with the substrate so that the formation of a hydranth is hindered (Torrey, 1910b); or if a ligature be tied about the piece, thus isolating the upper from the lower end (Torrey, 1910b; *cf.* Morgan, 1906); or if the primordium of the upper end be cut off a few hours after regeneration has begun (Watanabe, 1935), then the lower end does just one thing —it promptly forms a perfect hydranth.

These facts can only mean that somehow during development the field of the upper end encroaches on the field of the lower end and interferes with the processes of regeneration taking place there. It is either the severity of this interference of the upper end with the lower end, or it is the time (in the development of the lower end) at which this interference reaches the lower end which decides whether the lower end shall form a hydranth, a base or perchance nothing at all (Gilchrist and Schmid, 1930). We may think of the two ends as two armies seeking to entrench themselves and throw up fortifications. (The fortifications are, of course, the hydranths.) The army of the upper end has from the start the advantage of, let up say, higher ground and a quicker start. It is thus able to entrench itself without opposition from the lower end. The army of the lower end, on the other hand, soon finds itself hard pressed. If it is able to hold its own long enough, it may form a perfect hydranth, although the

hydranth which it forms is usually smaller than the hydranth formed by the upper end. If it is not able to hold its own, it may produce a secondarily incomplete hydranth; or it may completely change its plan of action and resort, as it were, to guerilla warfare; that is, it may form a base. (The metaphor of guerilla warfare is even more apt in the case of Tubularia, in which the lower end forms a mobile stolon of indeterminate growth.)[13]

Why does the lower end sometimes form nothing at all? Is this due to the complete subordination of the lower end by the upper end? No, for even the side of a piece close to a hydranth, and hence at least partially subordinate to the hydranth, will form a base. In typical monapical regenerates the lower end persists for a long time without visible differentiation. We must therefore seek further for an explanation.

(5) The phenomenon of *secondary incompleteness* (Fig. 4) is instructive in this connection. It will be recalled that in biapical regenerates the lower hydranths are often smaller than the upper hydranths, and are sometimes incomplete. When this happens it is the distal levels of the lower hydranths which are most reduced in size or are lacking. Now, it is to be noted that each regenerating piece has always at least one perfect distal end; namely, the distal end of the upper hydranth. The reduction in the lower hydranth, therefore, has something to do with the dominance of this upper distal end.

As a hypothesis we may assume that the field of the upper end pushes entirely across the length of the piece toward the field of the lower end. Presumably it pushes the field of the lower end back, and may even go so far as to obliterate or reverse it. Indeed, in Watanabe's (1935)

[13] In the "warfare" between the ends, the relative superiority of the upper end is of more consequence than the distance which separates the two ends. Thus the lower end of a long piece is more effectively subordinated than the lower end of a short piece, because in the long piece the difference in activity between the two ends is greater. In Tubularia the dominance of the upper end over the lower end is temporary; and after some hours the control of the upper end is withdrawn, and the lower end becomes free to develop a hydranth (Morgan, 1905).

observations on the progress of reduction in pieces stained with methylene blue, the encroachment of the upper field upon the lower field is practically rendered visible. Under these circumstances the invisible differentiations which are taking place at the lower end are disturbed. They seek to adjust themselves to the anomalous situation, and in doing so may form proximal tentacles distally. The phenomenon of secondary incompleteness, therefore, is of the nature of an abortive attempt to complete the differentiation of the zones of a hydranth in a field which has become reversed during the course of that differentiation.[14]

(6) A similar explanation may be applied to the phenomenon of *monapical regeneration.* There are in reality two sorts of monapical regenerates. In one, very short pieces of stem produce a single hydranth which never forms a stem or base because its lower end is differentiated as the proximal portion of a hydranth (primary incompleteness). In the other, longer pieces do not form a hydranth at the lower end because the lower end has differentiated and remains differentiated as the proximal portion of a secondarily incomplete hydranth. Figures 2e, 4a, 4b, 4c and 2g thus form a single unbroken sequence. In favor of this view is the observation that in cases of monapical regeneration the stem sometimes tapers to its apparently undifferentiated lower end, as it would toward a hydranth. (The development of a base from the side of a monapical regenerate has been several times observed.)

CONCLUSION

We are now in a position to answer the question as to how Corymorpha can be at the same time a realization and an achievement; a system in equilibrium and the product of a history lived. The process of *organization*

[14] The shift of the zone of proximal tentacle differentiation to the tip of the lower end is very significant. It suggests that the early phases of invisible differentiation are diffuse and even overlapping, and that the final localization of the definitive organ rudiments occurs relatively late in the process of invisible differentiation.

is realization. The gradient-field is labile and self-adjusting like a candle-flame. It has always the aspect of wholeness and ever tends toward an equilibrium within itself and with its surroundings. It is a true Gestalt. *Invisible differentiations on the other hand are achievement.* They are straightforward and creative processes. Like skyrockets they burst into an expanding pattern and fade away. Like a flower bud, they unfold into a blossom; and an injury to the bud must result in imperfection in the blossom. Indeed, the criterion of invisible differentiation is this capacity of a part to differentiate as a part. The organism is thus both a realization and an achievement, because in the process of its making it undergoes both organization and differentiation.

As students of regeneration we have been concerned principally with the processes of organization. We have cut the living material into various patterns and exposed it to the environment in various ways, in order to see how it will adjust itself to unnatural conditions. As our reward we have witnessed the plastic flame of the gradient-field adapting itself to form and forces. As experimenting embryologists we have taken material already in process of development and have operated upon it. We have discovered for the most part the increasing evidence of its invisible differentiation (localization, segregation, determination). We have thus been impressed with the straightforward, irreversible nature of ontogenetic processes. Now, both view-points are but abstractions from the total process of structurization. The organism which develops is neither pure Gestalt nor true history, because it is both. It is Gestalt first and fundamentally, because within its substance there lives a simple, labile and unified field or flame. Secondly, and on top of this, it is irreversible history because within this field localized processes of differentiation take place, and an ever-increasing manifoldness is created.

Summary

Let us now summarize the argument:

Biologists have vacillated between the concepts of the organism as a system pursuing an irreversible history and as a system tending toward an equilibrium (a Gestalt). In seeking to choose between these two points of view, the hydroid *Corymorpha palma* was cut transversely into a series of pieces. It was found (a) that every part had some power of reconstitution; but that (b) no two parts had the same power. This polyp is therefore both a system seeking equilibrium and the product of an irreversible history past.

A further analysis of certain of the phenomena of regeneration; namely, (1) primary incompleteness (the formation of proximally imperfect hydranths from very short pieces of stem); (2) proximo-distal regeneration (the capacity of the cut surface of a hydranth to reform parts distal to itself); (3) polarity (the tendency of the opposite ends of a piece to regenerate differently); (4) variability of the lower end (which may form either a hydranth, base or nothing at all); (5) secondary incompleteness (the formation of distally imperfect hydranths at the lower end of a piece of stem); and (6) monapical regeneration (the apparent production of nothing at the lower end of a piece) has led to the view that the structurization of a hydranth passes through three phases; A, *organization,* by which a simple, labile gradient-field is established within the piece, giving unity to the piece; B, *invisible differentiation,* by which different zones acquire definite and diverse capacities for development; and C, *visible differentiation,* in which the acquired potencies become patent. Of these the process of organization conforms to the principles of a system tending toward equilibrium (a Gestalt), while the processes of differentiation are straightforward and creative; (*i.e.*, true history).

LITERATURE CITED

Burr, H. S., and F. S. C. Northrop
1935. *Quart. Rev. Biol.*, 10: 322–333.
Child, C. M., and L. H. Hyman
1926a. *Biol. Gen.*, 2: 355–374.

Child, C. M.
1926b. *Ibid.*, 2: 609–630.
1926c. *Ibid.*, 2: 771–798.
1927a. *Jour. Exp. Zool.*, 47: 343–383.
1927b. *Ibid.*, 48: 197–221.
1928. *Physiol. Zool.*, 1: 419–461.
1932. *Ibid.*, 4: 593–627.
1935. *Jour. Exp. Zool.*, 71: 375–387.
Child, C. M., and Y. Watanabe
1935. *Physiol. Zool.*, 8: 395–416.
Gilchrist, F. G., and L. K. Schmid
1932. *Anat. Rec.*, 54: 55.
Huxley, J. S., and G. R. DeBeer
1934. "The Elements of Experimental Embryology." Cambridge. 514 pp.
Milojević, B. D.
1924. *Arch. f. Entw-mech.*, 103: 80–94.
Milojević, B. D., et B. Vlatkovic
1926. *C. R. Soc. Biol.* (*Paris*), 94: 685–687.
Morgan, T. H.
1904. Am. Nat., 38: 502–505.
1905. *Jour. Exp. Zool.*, 2: 495–506.
1906. *Ibid.*, 3: 501–515.
Peebles, Florence
1931. *Physiol. Zool.*, 4: 1–35.
Przibram, Hans
1924. *Arch. f. Entw-mech.*, 48: 203–444.
1931. "Conneeting Laws in Animal Morphology." University of London Press. 62 pp.
Torrey, H. B.
1910a. *Univ. of Calif. Publ. Zool.*, 6: 205–221.
1910b. *Biol. Bull.*, 19: 280–301.
Watanabe, Y.
1935. *Physiol. Zool.*, 8: 417–441.
Weimer, B. R.
1928. *Physiol. Zool.*, 1: 183–230.
Weiss, Paul
1926a. *Arch. f. Entw-mech.*, 107: 1–53.
1926b. "Morphodynamik. Ein Einblick in die Gesetze der organischen Gestaltung an Hand von experimentellen Ergebnissen." *Abh. zur theoretischen Biol.*, Heft 23.
1927. *Zeitschr. f. induk. Abst. u. Vererb.*, 46: 39–40.

GENETIC NATURE OF SPECIES DIFFERENCES[1]

DR. TH. DOBZHANSKY
W. G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY

INTRODUCTION

THE boundless diversity of organic forms is impressive even to a casual observer. Reducing this seemingly chaotic multiformity to some sort of rational system was a prerequisite for a scientific study of living things; hence taxonomy and morphology were in the past the most active branches, description and generalizing induction the main methods of biology. Later the main trend of thought has turned from morphology to physiology, from description to experiment, and from peculiarities of single species to properties common to large groups or to all of them. The problem of organic diversity must now be studied in a new aspect, namely, as a general property of living matter, for such it truly is.

The difference between any two individuals or species may be attributed to the differences between their gene complexes, and in a few cases between their cytoplasms. Assuming this statement to be correct (and it is not universally accepted), one must nevertheless admit that it does not represent an adequate solution of the problem of organic diversity, for it disregards the fundamental fact that the living world is subdivided into discrete groups of forms which we call species. The living world is not an array of individuals embodying all the possible combinations of the existing genes; it is certain that only an infinitesimal fraction of the possible gene combinations has been ever realized. Organisms are more or less adapted to their environment, and the gene patterns each of them carries must represent at least a tolerably harmonious whole. With the sexual process being the predominating mode of reproduction, an unlimited inter-

[1] Lecture delivered before the Genetics Society of America and Marine Biological Laboratory at Woods Hole on September 3, 1936.

breeding of all organisms would result, due to the properties of the Mendelian mechanism of inheritance, in a breakdown of the existing gene patterns and emergence of an almost infinite mass of recombinations. Among these recombinations some would be as harmonious as the existing ones, some might be even more so, but it is at least a fair guess that a vast majority would be discordant. Hence maintenance of life is possible only if the gene patterns whose coherence is tested by natural selection are prevented from disintegration due to unlimited hybridization. It follows that there must exist discrete groups of forms, species, which consist of individuals breeding *inter se*, but prevented from interbreeding with individuals belonging to other groups of similar nature.

On the other hand, evolutionary progress is possible only if new gene patterns are constantly being formed, since only by a process of trial and error can the always precarious balance between an organism and its environment be maintained. Mutation and sexual reproduction are the mechanisms that supply a store of new genic patterns. The process of evolution may then be described in a most general way as a result of the interplay of forces tending toward fixation of the already tested gene patterns, and forces producing new gene patterns some of which may become the forerunners of the world to come. One of the tasks of genetics is to secure an understanding of these forces and their interactions (*cf.* Wright, 1931).

It is a remarkable fact that in different organisms causes preventing free interbreeding of species are frequently different; isolation of species from each other is accomplished in nature by different means. Moreover, taking a given pair of species it is not uncommon to find that their interbreeding is averted not by a single but by several causes reinforcing each other's action. The expression "isolating mechanisms" seems to be a convenient general name for all the mechanisms hindering or preventing the interbreeding of racial complexes or spe-

cies. The present article gives an account of the isolating mechanisms found in three species of Drosophila, namely, *D. pseudoobscura* "race" A, *D. pseudoobscura* "race" B and *D. miranda*. The two "races" of *D. pseudoobscura* are very closely related; they seem to be morphologically identical, but can be distinguished because when crossed they produce sterile hybrids, and because they differ in a number of cytological and physiological characteristics (Lancefield, 1929, Dobzhansky, 1935, and others).[2] *D. miranda* differs from either "race" of *D. pseudoobscura* in a set of slight morphological characters, and also by its cytological and physiological properties (Dobzhansky, 1935). The discrimination of these species must be made through laboratory studies on living materials.

GEOGRAPHICAL ISOLATION

The geographical distribution of the three species under consideration is now known with a fair degree of accuracy (see map in Sturtevant and Dobzhansky, 1936). *Drosophila pseudoobscura* race B inhabits the country from British Columbia to California, and from the Pacific to the eastern slopes of the Sierra Nevada-Cascades mountain range. The distribution of *D. pseudoobscura* race A is much wider: from British Columbia to southern Mexico, and from the Coast Ranges (in the North) and the Pacific (in the South) to the Rocky Mountains and the western edge of the prairies. The coast of British Columbia, Washington, Oregon and northern California is inhabited by race B alone (race A reaches the coast only at Puget Sound, southern California, and possibly at the mouth of Columbia River). East of the Sierra Nevada-Cascades and southward from California race A only is found. The two races occur together in the southern Coast Ranges, in Sierra Nevada, and between the northern

[2] It is solely because of the lack of externally visible distinctions that these two forms are described as races of the same species. By any other criterion they should be considered distinct species, as the reader can see for himself on the basis of the data presented below.

Coast Ranges and the Cascades. It follows that although the areas inhabited by the two races are clearly different, the geographical isolation is far from complete; they occur together in so broad a zone that if their interbreeding were not prevented by other isolating mechanisms (see below) a large hybrid population would result.

The area inhabited by *Drosophila miranda* is comparatively very small, comprising only the region around Puget Sound. Since both races of *D. pseudoobscura* are found in parts of this region, no geographical isolation between *miranda* and *pseudoobscura* may be said to exist.

Ecological Isolation

Ecological isolation is a condition in which species or races are restricted to different habitats within the same geographical area. Since occupation of different geographic regions by two species may be due to preferences exhibited by each of them to habitats found only in its own region, there may exist situations which may be classed either as a geographical or as an ecological isolation.

D. pseudoobscura as well as *D. miranda* lives in forests, and is not usually found in treeless or desert localities (although a row of trees along a dry streambed may be sufficient to maintain a small population). In the south the distribution of *D. pseudoobscura* is sharply discontinuous, being restricted mostly to islands of forest growing on sufficiently high mountain ranges. The combined distribution of race A and race B of *D. pseudoobscura* is rather similar to, but wider than, that of the western yellow pine, *Pinus ponderosa*. Yet these flies are not bound to that tree only, and flourishing populations of either race were found in other coniferous (*e.g., Pseudotsuga*) or deciduous (*e.g.,* oak, aspen) forests.

Race A has a higher temperature optimum than race B (Dobzhansky, 1935), and the optimum for *D. miranda* is even lower than for race B. In view of this fact it is not surprising that in mountainous regions where both

races occur together race A occupies predominantly the lower and race B the higher elevations. Thus, a locality in the Kern River valley (California) lying at about 3,500 feet is inhabited by race A only, while on the tops of the surrounding mountains (Greenhorn Mountains, about 7,000 feet) a mixture of race A and race B with a predominance of the latter is found. This suggests a weak ecological isolation between race A and race B. *D. miranda* has been found thus far only in company with *D. pseudoobscura,* and the ecological preferences of the former are unknown.

SEXUAL ISOLATION

Lancefield (1929) observed that males of either race of *D. pseudoobscura* copulate with females of their own race sooner than with those of the opposite race. His results were corroborated by Mr. R. D. Boche working in our laboratory (unpublished). In Mr. Boche's experiments males were offered a choice of females of both races; several freshly hatched males of a given race were placed together with the same number of freshly hatched females of the same race and of females of the other race. At stated intervals of time some females were dissected, and the presence of sperm in their seminal receptacles was determined by microscopic examination. Boche found that at first males pair predominantly with females of their own race, but after the supply of the unfertilized females becomes small some interracial matings also take place. Thus, in one experiment race A males (Texas strain) were kept with race A (Texas) and race B (Seattle–4) females for 72 hours at 25° C.; 93 per cent. of the former and 19 per cent. of the latter females were fertilized. A pronounced sexual isolation between race A and race B is therefore established. On the other hand, no indication of even slight isolation was observed by Boche between strains of the same race coming from different geographic localities.

An aversion to mating with individuals of another species is clearly apparent also in crosses between *D. miranda*

and either race of *D. pseudoobscura*. In a series of experiments conducted by the present writer batches of five females and five males from the same strain of *D. pseudoobscura* and five females of *D. miranda* were kept together in the same vial for approximately 96 hours (at 21–23°). The presence of sperm in the seminal receptacles of these females was subsequently determined by dissection and microscopic examination. Among 376 *D. pseudoobscura* females 351, or 93.4 per cent., were found fertilized, while among 377 miranda females only 83, or 22.0 per cent., were fertilized. In another series of experiments five females and five males of *D. miranda* and five females of *D. pseudoobscura* were kept together in vials for 96 hours. The examination showed that 40.0 per cent. of miranda and only 13.75 per cent. of pseudoobscura females contained sperm (the totals of the flies dissected are 235 miranda and 240 pseudoobscura). In a third series of experiments *D. pseudoobscura* females were kept for nine days with *D. miranda* males, or *vice versa*. Although in this case the possibility of mating with representatives of their own species was excluded, a large percentage of females remained unfertilized. An interesting detail is that different strains of *D. pseudoobscura* exhibit different degrees of aversion to mating with *D. miranda*. Thus, the Oaxaca–5 strain (from southern Mexico) mates with *D. miranda* rather easily, while the Seattle–4 strain (Washington) refuses to cross in almost 90 per cent. of cases. This result indicates that within the species *D. pseudoobscura* hereditary factors are present which affect the crossability of this species with *D. miranda*. The potential evolutionary importance of such factors is obvious; it is fair to guess that the sexual isolation between two incipient species may be built up as a result of summation of a number of genetic factors of this kind.

Mechanical Isolation

Discrepancies between the structure of the male genitalia of one species and the female genitalia of another

may render copulation of representatives of these species difficult or impossible. Since in insects the external genitalia are made of inflexible chitin, and since species very similar in appearance are sometimes clearly distinct in genitalic structures, entomologists are prone to ascribe a great significance to the mechanical isolation of species (Jordan, 1905). There is no doubt that the structure of the genitalia may make interspecific crosses difficult; for instance, copulation of *D. melanogaster* male with *D. pseudoobscura* female may result in failure of separation and death of both participants. There is, however, no experimental evidence to show that small differences in the genitalia frequently prevent crossing, and Kerkis (1931) has proved statistically that at least in some Hemiptera the genitalia are as variable within a species as are the external structures. The claims that genitalic differences are of paramount importance in isolating species are greatly exaggerated.

Races A and B of *D. pseudoobscura* have identical genitalia, hence mechanical isolation is out of the question in this case. The genitalia of *D. miranda* are identical in structure with those of *D. pseudoobscura,* but since the former species is generally larger than the latter, the absolute size of the genitalia is correspondingly different. Nevertheless, observations on the copulation of *D. miranda* and *D. pseudoobscura* seem to show that no mechanical difficulty is encountered. Any one having experience with Drosophila breeding knows how greatly the dimensions of these flies vary under the influence of culture conditions, but offspring can be obtained from matings in which parents are very different in size.

VIABILITY OF THE F_1 HYBRIDS

The isolating mechanisms reviewed above have the common property of tending to prevent the appearance of hybrid zygotes. The mechanisms that remain to be considered concern the hybrids already produced, and tend to handicap or to eliminate these hybrids from the

breeding populations of the parental species. The simplest mechanism of this class is lowering of the viability of the F_1 hybrids, which in extreme cases results in death of the latter before they reach the stage of sexual maturity (*e.g.*, the fish hybrids described by Moenkhaus, 1910, Newman, 1914, and others).

The F_1 offspring from the race A $\times$ race B crosses in *D. pseudoobscura* seem to be about as vigorous somatically as the non-hybrid individuals of either race, although some observations indicate that the male progeny of the A♀ $\times$ B♂ cross consists of individuals that tend to be small in size. The male offspring from the cross *D. pseudoobscura*♀ $\times$ *D. miranda*♂ are almost completely inviable, the sex-ratio being about 1♂: 200♀. The reciprocal cross produces males that are abnormal in appearance, sluggish and rather short lived. The viability of the hybrid females from either cross is higher than that of their brothers but lower than that of the parental species.

Hybrid Sterility

When crossed, race A and race B of *D. pseudoobscura* produce in F_1 fertile females and sterile males. The female hybrids can be back-crossed to males of either parental race; their daughters are all more or less fertile, while some of the sons are fertile and others are sterile (Lancefield, 1929). The cytological basis of the sterility of the F_1 males is a disturbance of the spermatogenesis: chromosome pairing is incomplete or absent, the first meiotic division abortive, the second division absent, degenerate polyploid cells are formed instead of spermatozoa. Although these disturbances are greater in crosses between some strains than between others, and although in any given cross the abnormalities are greater at high than at low temperatures, the derangement of the spermatogenesis is under all conditions so profound that the sterility of the F_1 males is complete (Dobzhansky, 1934). Preliminary studies on the spermatogenesis in back-cross males show a whole gamut of conditions, ranging from

normal to even greater disturbance than that observed in F_1 males. The hybrids between *D. miranda* and either race of *D. pseudoobscura* are sterile in both sexes. In the males testes are vestigial, while females deposit eggs which produce no larvae. Several studies of the causes of the sterility of the above hybrids have been made; only a summary of the results obtained may be presented here.

In general, hybrid sterility may be due to several causes. Perhaps the most thoroughly studied case of sterility, that of the hybrids between the European and the Japanese races of *Lymantria dispar* (Goldschmidt, 1934), is due to a lack of balance between the sex-determining factors coming from the parental races. The sterile hybrids are here intersexes. That the *D. pseudoobscura* A $\times$ B, and the *D. pseudoobscura* $\times$ *D. miranda* hybrids are not intersexes follows from the fact that their secondary sexual characters, as well as their reproductive systems except the gonads, are normal (Dobzhansky and Boche, 1933). The writer has recently found several individuals of race A *D. pseudoobscura* which were probably (and one of them certainly) triploid intersexes. Their reproductive organs were quite different from those of the sterile hybrids.

Another possible cause of hybrid sterility is dissimilarity of the gene arrangement in the chromosomes of the parental forms (chromosomal sterility). In a number of cases, especially among plants, it is now established that races and species may differ in gene arrangement. The first case in which a difference of this sort has been found is that of *D. melanogaster* and *D. simulans* (Sturtevant and Plunkett, 1926, an inverted section in one of the chromosomes). Tan (1935) and Koller (1935) have shown that race A and race B of *D. pseudoobscura* differ in four inverted sections (two in the X-chromosome, one in the second and one in the third chromosomes). The chromosomes of *D. miranda* are built very differently from those of *D. pseudoobscura* (Dobzhansky and Tan, 1936, and a paper in press). In all the chromosomes many genes have

changed their relative locations due to inversions. Some genes located in the same chromosome in one of these two species are located in different chromosomes in the other, indicating that translocations have taken place in the phylogeny. Each species has chromosome sections that can not be identified with certainty in the other; these sections may be accounted for either on the supposition that losses of genic materials have taken place in the phylogeny, or on the supposition that certain parts of the chromosomes were subject to so many reorganizations of the inversion or translocation types that they are no longer identifiable with the aid of the salivary gland chromosome method which was employed in these studies. Dobzhansky and Tan estimate that in order to derive the gene arrangement observed in *D. miranda* from that present in *D. pseudoobscura,* or *vice versa,* at least forty-eight, and probably many more, chromosome breakages and reattachments must take place.

Granting that some, and possibly all, species differ from each other in gene arrangement, one nevertheless must be circumspect in attributing the hybrid sterility to this cause. Cumulative effects of rearrangements of genic materials within the chromosomes may lead to a situation where meiotic pairing between the original and the altered chromosomes will be mechanically difficult or impossible; this, in turn, may result in disturbances at disjunction, and in production of gones containing unbalanced chromosomes complements. In plants such gones are frequently inviable, and thus sterility of a hybrid may result. It is, however, not obvious how such a mechanism can produce complete sterility, since even if chromosomes disjoin at meiosis entirely at random a few gones containing balanced chromosome complements should be produced. Hence, accessory hypotheses are needed to explain the complete absence of functional gones in many sterile hybrids. An additional, and even more serious, difficulty is met with if the sterile hybrids in animals are considered. For it is known that, at least in Drosophila,

gametes carrying even grossly unbalanced chromosome complements remain functional in fertilization. Translocation heterozygotes and triploids in animals produce functional gametes, some of which may give rise to inviable zygotes. Yet, sterile hybrids in animals, just as in plants, are characterized by non-production of functional gametes rather than by production of inviable zygotes. The writer believes that thus far no case of sterility either in plants or in animals has been conclusively proved to be chromosomal in nature. The main argument that chromosomal sterility exists at all is the occurrence of fertile allopolyploids derived from sterile diploid hybrids, but this argument is not necessarily decisive.

The sterility of the hybrids between race A and race B of *D. pseudoobscura* is certainly not chromosomal, as shown by the following evidence. (1) The inverted sections found in the chromosomes of these hybrids are too few to produce sterility; fertile individuals heterozygous for a larger number of inversions can be obtained artificially in *D. melanogaster*. (2) The hybrids between some strains of *D. pseudoobscura* show complete chromosome pairing at meiosis and yet they are sterile. (3) In the hybrids abnormalities in spermatogenesis are observed at stages preceding as well as following meiosis. (4) Reduplication of the chromosome complement (allotetraploidy) in a section of the testis in the F_1 males does not alter the course of the spermatogenesis (Dobzhansky, 1933, 1934). On the other hand, it has been shown that the sterility of the hybrids under consideration is genic in nature, that is dependent upon interactions of complementary genes contributed by both parents (Dobzhansky, 1936). Such "sterility genes" are present in all the chromosomes of each race studied, and in the parts of the chromosomes having different gene arrangement in the two races, as well as in the parts in which the gene arrangement is similar. The back-cross males are sterile or fertile, depending upon which combination of the chromosomes of the ancestral races they carry. The precise mechanism through

which the sterility genes exert their action leading to the disturbance of the spermatogenesis in the hybrids is unknown, but this mechanism is probably intracellular in nature. This is suggested by the experiments of Dobzhansky and Beadle (in press), who transplanted testes of the hybrid males into males of the pure races and *vice versa*, observing that in all cases the development of the implants as well as of the host's testes proceeds autonomously, *i.e.*, in accordance with their own genetic constitution.

The cause of the sterility of the *D. miranda* × *D. pseudoobscura* hybrids is unknown at present. The profound differences in gene arrangement observed between these species warrant a suspicion that in these hybrids chromosomal sterility, or a combination of chromosomal and genic sterility, may be involved, but further studies are needed to elucidate this point (*cf.* Dobzhansky and Tan, 1936).

Viability in the F_2 and in Further Generations of Hybrids

As stated above, the F_1 hybrids from the cross *D. miranda* × *D. pseudoobscura* are completely sterile, and no F_2 generation can be obtained. The F_1 hybrid females from A × B crosses in *D. pseudoobscura* are, however, fertile, and back-cross progenies can be produced. Lancefield (1929) has noticed that in these back-cross progenies the sex-ratio is distorted in favor of females. Dobzhansky and Sturtevant (1935) have confirmed this observation, and pointed out that the general viability of the back-cross products is very low in comparison both with the pure races and with the F_1 hybrids. Some flies of either sex are visibly weak and show various somatic abnormalities, many of the females are either completely sterile or produce very few offspring, the longevity of the flies is generally low. This weakness is more pronounced among males than among females, which fact accounts for the modification of the sex-ratio.

Some of the individuals obtained from the back-crosses must have all the chromosomes, and hence all the genes,

of the race to which the father of the back-cross belongs; other individuals are identical in chromosomal constitution with the F_1 hybrids; but the majority of individuals carry various combinations of the chromosomes of race A and race B. It was tempting to suppose that the low viability of the back-cross products is due to unfavorable effects of mixtures of chromosomes of the two races, that is to say, that individuals carrying some chromosomes of one race and other chromosomes of the other race have an inferior viability. A closer study has shown, however, that this guess is not true, or at any rate not adequate to account for the whole complex of facts (Dobzhansky and Sturtevant, 1935, and unpublished data).

Experiments were so arranged that it was possible to determine the racial origin of all the chromosomes (except the very small fifth chromosome) present in a given back-cross individual by inspection of its phenotype. For this purpose strains of race A and race B having the chromosomes marked by appropriate mutant genes were intercrossed, and the resulting F_1 females were back-crossed to males of both parental races. The startling result of these experiments was the fact that those individuals in the back-cross progenies that are identical in their chromosomal constitution with individuals of pure races or with the F_1 hybrids proved to have a low viability, just as low as the individuals carrying various mixtures of the chromosomes of both races. In other words, the low viability observed in the back-crosses between race A and race B is general, and not restricted to some classes carrying particular combinations of chromosomes. The only way to account for this situation is to suppose that the low viability of the back-cross products is due to a maternal effect, that is, to an influence exerted by the chromosomal constitution of the mother on the development of her eggs. The F_1 females from the interracial crosses carry half of the chromosomes of race A and another half of race B; it appears that the presence of this hybrid chromosome complement in the developing oocyte (or in

the surrounding tissues) influences the constitution of the resulting egg in such a way that the viability of a zygote coming from this egg is decreased. Furthermore, this decrease of viability is independent of the chromosomal constitution which the eggs possess after reduction and fertilization, in the sense that individuals having the same chromosomal constitution are less viable if they come from eggs deposited by an F_1 female than if they develop from eggs of a pure race mother. Thus, an individual carrying all race A chromosomes obtained in a back-cross of an F_1 hybrid female to a race A male is greatly inferior in viability to an individual of pure race A parentage.

On the other hand, the offspring of a given F_1 hybrid female may be more or less viable, depending upon the male to which she is mated. Thus, in one experiment made by the present writer race A females carrying the genes beaded, yellow, vermilion, singed and short were crossed to race B males carrying the genes scutellar and prune. The resulting F_1 females were back-crossed to race A beaded yellow vermilion singed short males. About one hundred culture bottles of this back-cross have produced not a single adult offspring; an inspection of the bottles has shown, however, that many eggs were deposited in them, but that the larvae coming from these eggs have died in very early stages. The decrease of the viability observed here is, consequently, so great that all the back-cross zygotes die before reaching maturity. Nevertheless, the same F_1 females proved capable of producing relatively more viable offspring. They were separated from their mates and re-crossed to wild-type race A males (the Texas strain); larvae soon appeared in the cultures, and at least some of them grew to maturity and produced adults. This experiment has been repeated twice with identical results, and moreover some other experiments involving different strains of A and B races have behaved similarly.

The phenomena of maternal effects have considerable interest intrinsically, and further experiments in this field

are in progress. At present we are interested in this subject only in so far as it has a bearing on the problem of isolating mechanisms. The good viability of the F_1 generation of the interracial hybrids stands in sharp contrast to the low viability of the offspring of the F_1 hybrid females. It is safe to assume that under the conditions of competition in nature this deterioration of viability will tend to eliminate the interracial hybrids from the breeding populations of the ancestral races.

SUMMARY AND CONCLUSIONS

The interbreeding of race A and race B of *Drosophila pseudoobscura* is impeded by (1) a pronounced, though incomplete, geographical isolation, (2) a weak ecological isolation, (3) a marked sexual isolation, (4) a complete sterility of the F_1 hybrid males and of a part of the back-cross males, and (5) a low viability of the offspring of the back-crosses of the F_1 hybrid females to males of the parental races. None of these isolating mechanisms is in itself sufficient to achieve a complete separation of the breeding populations of the two "races," but taken together they probably accomplish this task with a margin of safety.

The interbreeding of *D. miranda* with either race of *D. pseudoobscura* is precluded by (1) a strong sexual isolation, (2) a decrease of the viability and (3) complete sterility of the F_1 hybrids of both sexes. The last of these isolating mechanisms is sufficient for a total separation of the two species, the others increasing the margin of safety.

The great variety and the apparent high efficiency of the mechanisms isolating the two "races" of *D. pseudoobscura* from each other is rather surprising, since these "races" seem to be, at least judging by their external similarity, very closely related. No indication that interracial hybrids occur in nature has been found. In other groups of organisms, notably in some families of plants, the isolation of species is by no means so secure, and hy-

bridization of species in nature is frequently reported in literature. The significance of such differences in the behavior of different groups of organisms is unknown at present, but it seems certain that they must exert a profound influence on the evolutionary pattern of a given group.

The mechanisms isolating species from each other must be considered the only true specific characters, if the expression "specific character" is to have any real meaning. The genetics of species differences is therefore a study of the hereditary nature of the isolating mechanisms, and of their rôle in the dynamics of Mendelian populations.

It has been contended by many authors that the grouping of individuals into species is merely a matter of convenience, since species have no existence apart from the mind of investigator. As a proof of this contention, it has been pointed out that such criteria of species distinction as the production of sterile hybrids sometimes break down because some forms which are classed as species can be crossed experimentally and can produce semi-fertile or fertile hybrids. This point of view is fallacious, and is based on a failure to understand that the fact that some species can be crossed and can produce fertile hybrids does not prove that these species cross regularly in nature. Species is a dynamic rather than a static entity, and the essential feature of the process of species differentiation is the formation of discrete groups of individuals which are prevented from interbreeding with other similar groups by one or more isolating mechanisms. Isolating mechanisms seem to be a rather haphazard collection of phenomena, and yet their genetic effects are alike in kind, namely, the formation and maintenance of discrete groups of organisms. The degree of isolation of these groups from each other is of necessity variable, presumably increasing with time, but in some cases perhaps also receding and disappearing. A thorough understanding of the nature and the functioning of isolating mechanisms is essential, because without it no trustworthy picture of the mechanism of evolution can be drawn.

LITERATURE CITED

Dobzhansky, Th.
1933. *Proc. Nat. Acad. Sci.*, 19: 397–403.
1934. *Zeits. Zellf. mikr. Anat.*, 21: 169–223.
1935. *Genetics*, 20: 377–391.
1935. *Jour. Exp. Zool.*, 71: 449–464.
1936. *Genetics*, 21: 113–135.
Dobzhansky, Th., and G. W. Beadle
1936. *Genetics* (in press).
Dobzhansky, Th., and R. D. Boche
1933. *Biol. Zentralblatt*, 53: 314–330.
Dobzhansky, Th., and A. H. Sturtevant
1935. *Proc. Nat. Acad. Sci.*, 21: 566–570.
Dobzhansky, Th., and C. C. Tan
1936. *Zeits. ind. Abst. Vererbungsl.* 72: 88–114.
1936. AM. NAT., 70: 47–48.
Goldschmidt, R.
1934. *Bibliogr. Genetica*, 11: 1–186.
Jordan, K.
1905. *Zeits. wiss. Zool.*, 83.
Kerkis, J.
1931. *Zool. Anzeiger*, 93: 129–143.
Koller, P. Ch.
1936. *Jour. Genetics*, 32: 79–103.
Lancefield, D. E.
1929. *Zeits. ind. Abst. Vererbungsl.*, 52: 287–317.
Moenkhaus, W. J.
1910. *Proc. Indiana Acad. Sci.*, 353–393.
Newman, H. H.
1914. *Jour. Exp. Zool.*, 16: 447–499.
Sturtevant, A. H., and Th. Dobzhansky
1936. In press, AMER. NAT.
Sturtevant, A. H., and C. R. Plunkett
1926. *Biol. Bull.*, 50: 56–60.
Tan, C. C.
1935. *Genetics*, 20: 392–402.
Wright, S.
1931. *Genetics*, 16: 97–159.

SHORTER ARTICLES AND DISCUSSION

VARIATION IN SCUTES AND PLATES IN THE BOX-TURTLE, *TERRAPENE CAROLINA*

A NUMBER of writers have noted the fact that the chelonians in general, and particularly some species, are subject to a considerable amount of variation in the number and arrangement of the horny scutes which cover the shells of these animals. Such abnormalities have been variously interpreted as results of injury (Knoll, 1935), as possible indications of progressive steps in evolution (Berry, 1935) and as atavistic recurrences of scutes that have been lost in the course of phylogeny (Gadow, 1899, 1905, Newman, 1906). Five specimens of *Terrapene carolina* (Linnaeus) in the writer's collection show supernumerary scutes on the carapace and present some unusual conditions which seem worthy of record in this connection. They were all collected from the same general locality in the region of Onville, Stafford County, Va., during the summers of 1927 to 1930. The first two of these which will be discussed are given only brief consideration, since the variations which they exhibit are of rather common occurrence; the other three, however, are of considerably more interest and are described in greater detail.

The normal scutellation of the carapace in this species consists of: a row of five large median shields, the vertebrals; two lateral rows of costals, with four shields in each row; and a row of marginals, forming the borders of the shell. The marginals, of which there are twelve pairs, are small and are separated anteriorly by the tiny nuchal shield. The first specimen to be described (J. H. U. Mus. No. 33) is the carapace of a juvenile individual 86 mm in length. It differs from the above scheme in the possession of an extra shield lying at the posterior end of the costal series on the left side. This supernumerary scute, which thus separates the fourth left costal from the last vertebral, could be interpreted as a fifth left costal. However, the normal costals of the two sides are about equal in size, and it therefore seems probable that the extra shield should more properly be regarded as the product of a division of the fifth vertebral.

The second specimen (J. H. U. Mus. No. 114) is an adult male. An extra scute is inserted between the fourth and fifth vertebrals, and another, adjacent to it, separates the third and fourth left

costals (Fig. 1). The lines of growth on the shields are very well-marked in this specimen, and it is easily demonstrable that the supernumerary scutes are of the same age as the normal ones and could not have been formed as the result of injury or division late in life.

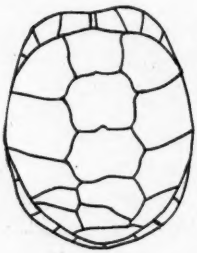


FIG. 1

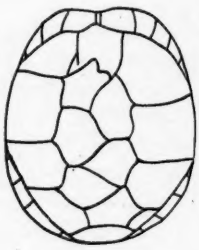


FIG. 2

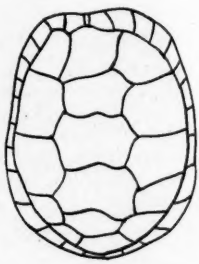


FIG. 3

Such asymmetrical variations in the costals as the two just described are not infrequently encountered and a large number of cases has been recorded in the literature for different species (Gadow, 1899; Newman, 1906; Coker, 1905, 1910). J. H. U. Mus. No. 16, an adult female, is of more interest than these, however; for while it exhibits normal scutellation in both the vertebral and costal series, symmetrically placed supernumerary marginals are present. There are thus thirteen pairs of marginals instead of twelve pairs. Comparison with normal specimens shows that this increase has occurred at the posterior end, for in this individual the fifth vertebral contacts six marginal shields instead of the usual four. Unfortunately it is not possible to ascertain whether a corresponding increase in the number of peripheral bones has occurred in this turtle. On the basis of the records of Parker (1901) and of Newman (1906) one would expect this to be the case.

A fourth specimen (J. H. U. Mus. No. 69), an adult female, shows a very interesting duplication of vertebrals. It possesses eight vertebral scutes instead of five, and these are arranged in a more or less staggered series, as shown in Fig 2. Several specimens of this general type have been described by other writers and have formed the basis for some theoretical discussion. Gadow (1899) considers such an arrangement of extra vertebrals as evidence pointing toward the fact that the vertebral series was originally paired, but Newman (1906) seems inclined to regard it as a more or less fortuitous occurrence resulting from crowding

of the linear members of the row. Coker (1910), on the other hand, points out that in such specimens the normal alternation of vertebrals and costals is still maintained; the line of separation between two costals always falls opposite the middle of the adjacent vertebral just as in normal specimens. He therefore remarks that "there is observed an adaptation between asymmetrical neurals (vertebrals) and asymmetrical costals that strongly suggests that the explanation of these scutes is to be sought in the *adjustment* of scutes consequent on some more primary asymmetry" and says, "I do not regard them as belonging in linear sequence and crowded out of position."

The last specimen to be discussed (J. H. U. Mus. No. 30) has extra scutes in all series; there are six vertebrals, five pairs of costals and fourteen pairs of marginals arranged as shown in Fig. 3. This individual thus possesses one extra vertebral, paired supernumerary costals and two extra pairs of marginals. In addition, it also exhibits a variation in the bony plates which underlie the scutes. It has eleven thoracic vertebrae instead of ten, and nine pairs of ribs instead of eight pairs go into the formation of the carapace. The upper shell thus possesses nine neural plates and nine pairs of costals and is bordered by fourteen pairs of peripheral plates. As a result of the presence of these extra bones, the specimen appears longer and narrower than normal. The length of the carapace divided by the width gives a ratio of 1.32, while measurement of a number of normal specimens shows an average length-width ratio of 1.20 for females and 1.25 for males. The exact correspondence in the number of supernumerary bony plates and the number of supernumerary horny scutes in this specimen is of great interest. Both Newman (1906) and Coker (1910) have pointed out that there always seems to be a direct correlation between the peripheral plates and the marginal scutes. In all known cases of increase or decrease in the number of marginals, examination of the underlying bones has revealed a corresponding variation in the number of peripherals. However, this is by no means true for the vertebrals and costals. Extra scutes in these series often occur without any correlated plate abnormalities and, conversely, disturbances in the number or arrangement of the bony plates often leave the scutes unaffected. Wandolleck's (1904) description of a turtle in which lateral curvature of the spine had greatly disarranged the bony plates but had resulted in no abnormalities in scutellation is a case in point. Moreover, Newman (1906)

found that even when a correlation does exist, extra paired costal plates are usually associated with *asymmetrical* extra costal scutes rather than with symmetrical ones. The present case is thus of special interest, for it exhibits a direct correlation of scutes and plates in *all* series.

The possible significance of these variations for theoretical interpretations need not be discussed at length. This has already been done on the basis of much larger series of specimens by Gadow (1899, 1905), by Newman (1906) and by Coker (1905, 1910). It will suffice to say that supernumerary scutes are of rather common occurrence in the carapace of turtles. A brief search of the literature reveals that they have been recorded for at least the following American species: *Sternotherus* (*Aromochelys*) *odoratus* (Newman, 1906), *Chelydra serpentina* (Newman, 1906), *Clemmys guttata* (Coker, 1905), *Clemmys* (*Chelopus*) *insculpta* (Parker, 1901), *Terrapene carolina* (Newman, 1906; Berry, 1935), *Malaclemys centrata* (Coker, 1905, 1910), *Malaclemys pileata littoralis* (Hay, 1904; Coker, 1910), *Graptemys geographica* (Newman, 1906), *Chrysemys marginata* (Newman, 1906), *Chrysemys picta* (Newman, 1906), *Pseudemys* (*Ptychemys*) *elegans* (Agassiz, 1857), *Chelonia* (*Chelone*) *mydas* (Coker, 1905), *Caretta* (*Thalassochelys*) *caretta* (Gadow, 1899; Coker, 1905, 1910; Babcock, 1930). Variations in the number of plastral scutes, on the other hand, are rare. Newman describes a few cases in Chrysemys, Chelydra and Graptemys, and Coker (1910) found interplastrals and supernumerary plastrals as rare abnormalities in Malaclemys. Newman points out, however, that the species *Sternotherus* (*Aromochelys*) *odoratus* is a striking exception to this general rule and is subject to much variation in the plastral scutes.

A reduction in the number of scutes is of much rarer occurrence than is an increase in the number. Such losses, possibly the result of fusion, are recorded by Parker (1901), by Newman (1906), by Coker (1910) and by Knoll (1935). It is worth noting also that variations in the scutes of turtles seem to occur much more frequently at the posterior end than at the anterior end; and that, for Malaclemys at least, females show a much greater tendency towards variation than do males (Coker, 1910).

In conclusion it may be said that the author is in general agreement with Coker as to the significance of these variations. Careful examination of these specimens makes it obvious that most of the variations can not be accounted for on the basis of injury,

exposure to brush fires or deformity by disease. Coker's discussion seems to have adequately disposed of the "atavism" theory and to have shown that variations of this sort probably have no phylogenetic significance. It is more likely that they are of the nature of fusion or division of parts. This, however, would not eliminate the possibility of a direct correlation between plates and scutes. These structures are intimately related, the scutes being of epidermal origin, while the plates are, at least partly, dermal. In the marginal series there is a scute for every plate and it would not be at all surprising if, along with the appearance of an extra plate, a new center for formation of a scute should arise. In the vertebral and costal series, on the other hand, no such exact correspondence exists; four costal scutes are normally underlain by eight costal plates and five vertebral scutes overlie eight neural plates and two procaudals. This being the case, exact correlation of supernumerary plates and scutes in these regions would not be expected and is, in fact, not usually found. Where such correlations do seem to exist, as in the last case reported here, they are probably only indirect. For when extra vertebrae and ribs appear the carapace is unusually long, as has been pointed out, and it is likely that the extra scutes appear as a secondary result of this elongation, not as a primary result of the presence of extra bones.

W. GARDNER LYNN

THE JOHNS HOPKINS UNIVERSITY

LITERATURE CITED

Agassiz, L.
1857. "Contributions to the Natural History of the United States of America." Vol. II Boston: Little, Brown and Company.
Babcock, H. L.
1930. AMER. NAT., 64: 95–96.
Berry, C. T.
1935. *Sci. Monthly*, 40: 566–569.
Coker, R. E.
1905. *Johns Hopkins University Circ.*, 178: 9–24.
1910. *Jour. Morph.*, 21: 1–75.
Gadow, H.
1899. "Willey's Zool. Results," Part 3: 207–222.
1905. *Science*, n.s., 22: 637–640.
Hay, W. P.
1904. *Bull. Bur. Fisheries*, 24: 3–19.
Knoll, C. M.
1935. *Copeia*, p. 100.
Newman, H. H.
1906. *Biol. Bull.*, 10: 68–114.

Parker, G. H.
1901. AMER. NAT., 35: 17–24.
Wandolleck, B.
1904. *Zool. Jahrb.*, Bd. 20 S. 151–166.

SEGREGATION OF MUTANT CHARACTERS OF DEER MICE[1]

THE object of the study reported in this paper was the initiation of an analysis of the genetic relationships of the mutant characters which appear in the deer mice of the genus *Peromyscus*. Those reported upon here were the available characters which could be readily ascribed to single genes and which could be distinguished from the wild type with certainty after crosses between strains or races. Comparisons with similar characters of other species prove of interest, although considerable difficulty accompanies efforts in identification of homologous characters of this mouse and the other laboratory mammals. Albinism, pallid, hairlessness, dominant piebald and yellow were included in this study.

These characters, with the exception of the piebald, were described by Sumner (1932) in races of *Peromyscus maniculatus*. The piebald character occurred in a wild population of *P. maniculatus bairdii* in Illinois. The writer is grateful to Dr. Elmer Roberts for the foundation piebald mice and to Dr. Lee R. Dice for representatives of the four characters described by Sumner. Miss E. Lucile Beerbower, research assistant in vertebrate genetics, provided valuable assistance on the routine of the experiments.

Albinism in the deer mouse, as in other mammals, is a recessive Mendelian character in which there is an almost total absence of pigmentation in the eyes, skin and hair. The other color characters, consequently, can not make their phenotype manifest in homozygous albinos and are not classifiable by inspection in such animals. Pallid is a recessive Mendelian character which greatly reduces the dark pigments of the eyes, skin and hair but does not affect appreciably the yellow of the coat. Homozygous pallid mice have a pale, tawny yellow coat-color and red eyes. The character resembles very closely the pink-eyed and red-eyed dilutions of the house mouse and Norway rat. Color characters

[1] This is a contribution from the Laboratory of Vertebrate Genetics, University of Michigan. Grants from the Faculty Research Fund maintained the work in 1928 and 1929.

which reduce the black of the eyes and fur to lesser extents than does pallid or not classifiable in homozygous pallid animals. Yellow is also a recessive Mendelian character by which the black pigment of the eyes, skin and hair are slightly reduced, but the yellow is not visibly affected. Homozygous yellow mice appear to have black eyes and an almost clear yellow coat. Hairless, a Mendelian recessive character, appears in the deer mouse very much as in the house mouse and Norway rat. Homozygous hairless deer mice have a juvenile coat but usually fail to regenerate subsequent pelages. Color characters which affect the pigmentation of the coat only are therefore not classifiable in homozygous hairless animals after the juvenile pelage is lost. The piebald which was used in this work is a Mendelian dominant. The homozygous and heterozygous forms show a white area which usually involves all the head, parts of the ventral surface and most of the tail stripe. The area varies among individuals in shape and extent, but it is always sufficiently large to be readily identified.

The method used in this study was the very simple one usually applied in examining the segregation of two Mendelian characters for evidence of linkage. Individuals were produced which were heterozygous for each of the two character pairs to be tested together. Such double heterozygotes were then mated with mice, which were homozygous for the recessive member of each pair. The numerical proportions of the four types of young from these matings revealed the gametic ratio of the heterozygotes and consequently the presence or absence of random segregation. In the case of each test, with one exception (albinism *vs.* piebald), the double heterozygotes were produced in both of the possible ways; that is, both recessives from one parent (coupling) and one recessive from each parent (repulsion). The offspring of each type were, of course, recorded separately, since parental combinations of one were recombinations of the other. The two types of each test produced essentially the same proportion of recombinations, and they are not separated in Table I. The original plan of the work called for at least 500 young from the test of each character with each of the others. This was made impossible by an abrupt conclusion of the work. The numbers which were secured, however, and are presented in Table I are sufficient to indicate with some degree of certainty the segregation in each combination.

TABLE I
SEGREGATION OF MUTANT CHARACTERS OF DEER MICE

Characters tested	Total gametes tested	Observed recombinations	Per cent. of recombinations	Deviation / P.E.
Albinism *vs.* Pallid.....	365	82	44.93 ± 2.49	2.03
Albinism *vs.* Hairless.....	296	151	51.01 ± 1.96	.51
Albinism *vs.* Piebald.....	199	53	53.26 ± 3.37	.93
Albinism *vs.* Yellow	326	80	49.08 ± 2.64	.34
Pallid *vs.* Hairless	215	109	50.69 ± 2.30	.30
Pallid *vs.* Piebald	234	102	43.59 ± 2.20	2.91
Pallid *vs.* Yellow.....	194	55	56.70 ± 3.42	1.95
Hairless *vs.* Piebald	297	150	50.50 ± 1.96	.25
Hairless *vs.* Yellow.....	340	161	47.35 ± 1.83	1.44
Piebald *vs.* Yellow.....	274	131	47.81 ± 2.04	1.07

The above descriptions make obvious the fact that some combinations of the characters were not distinguishable. Thus albinism prevented the classification of other color characters in half of the classes of tests with it. In the tests of albinism with pallid, piebald and yellow, therefore, it was only possible to distinguish the colored recombination type from the colored parental type. The recombinations recorded in the table under these three tests include only the colored type. When the percentage of recombinations was calculated for these tests it was assumed that the albino recombination class was equal numerically to the colored. The probable errors were calculated on the basis of the numbers of colored mice only. Similarly, in the test of pallid with yellow the non-pallid individuals only were useful in determining the proportion of recombinations and were therefore used as the size of the sample in computing the probable error. The percentage of recombinations was calculated by assuming the recombinations in the pallid types to be the same as in the non-pallid. It was necessary to classify all litters in the tests of hairless with piebald and hairless with yellow before the age of thirty days, since after that age the absence of hair made the two types of hairless mice in each indistinguishable.

The results of the tests on the ten possible combinations of the five characters indicate that each segregates in a random manner with each of the others. The percentage of recombinations is in every case sufficiently close to fifty to be expected in random sampling when a linkage does not exist. It may be concluded, therefore, that the genes from which the characters albinism, pallid, hairless, piebald and yellow originate in Peromyscus are probably located in separate chromosomes. There is the alternative that two of them may have remote loci in one chromosome. In that case the frequency of crossing over might be sufficiently great to be confused with independent segregation in

this material. Additional mutations of other loci on that chromosome would be necessary to a final proof of the existence of such a situation. At present there is no material available in Peromyscus which can be made to give information on the behavior of the chromosomes in relation to crossing over.

The characters, albinism and pallid, reported upon in this paper, are very similar to characters of the house mouse and Norway rat, as noted above. In each of these rodents a linkage exists between the two characters, yet in Peromyscus they appear to be inherited independently. This apparent anomaly may be accounted for in two ways: One, in spite of similar effects on the rodents, albinism or pallid or neither is the homolog of the characters of the forms indigenous to Asia; the other, if these characters of Peromyscus, house mice and Norway rats are due to homologous genes, chromosome structure has changed in the American form since its departure from the common ancestor. The hairless of Peromyscus may prove to be inherited in a manner similar to the inheritance of the corresponding character of other rodents when suitable mutations arise for tests. This would strengthen the view that a homology exists. Piebald and yellow of Peromyscus bear only very superficial resemblances to corresponding characters of the other rodents and rabbits.

HORACE W. FELDMAN

HYDE PARK, MASS.

LITERATURE CITED

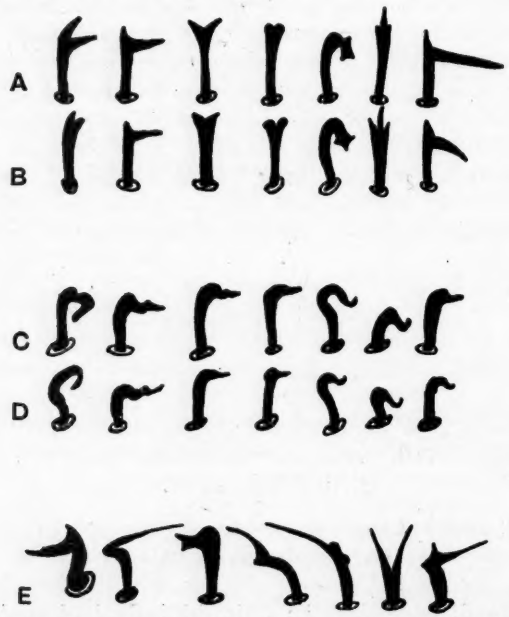
Sumner, F. B.
1932. *Biblio. Genetica,* 9: 1–106.

THE EFFECT OF COMPRESSION ON BRISTLE GROWTH IN WILD-TYPE SELF-IMPLANTS OF DROSOPHILA MELANOGASTER

DORSAL mesothoracic disks of mature wild-type larvae of *D. melanogaster* have been implanted in wild-type larvae of the same age by means of the technique recently described by Ephrussi and Beadle (1936). This disk is destined to give rise to the wing, half-thorax (except the humerus) and half-scutellum (Chen, 1929). All three regions can be readily identified in an implant when it is dissected out of the host fly. Nine thoracic and two scutellar bristles are formed by each disk. All these are identifiable in a perfect implant. In a previous paper dealing with the growth of wing-thoracic disks on implantation (Howland, Sonnenblick and Glancy, 1937), attention was called to the

production of bristle abnormalities in wild-type implants, but the full significance of this was not then emphasized.

From a new series of mesothoracic implants striking bristle phenotypes identical with those found on the mutants forked (f) and singed (sn^3) have been frequently obtained. In experiments involving the implantation of optic disks these two bristle types have also been noticed in the head bristles surrounding the eye cup. In addition to these, there are found simple bent bristles and a variety of forms which do not resemble those characteristic of any known mutant in *D. melanogaster*. Illustrated below are a number of bristle types found in implants and, to contrast and compare with them, are also shown similar bristles from the mutants forked and singed.



Bristles found in the mutant stocks forked and singed and in implants of wild type in wild type. A—from implants; B—from forked; C—from implants; D—from singed; E—from implants, various other types.

Of the factors which might be responsible for bristle distortion, it is apparent that the pressure exerted on the disk by the transfer pipettes should be considered. If pipettes of several sizes are used, some of which elongate the disks, others of which are sufficiently large to accommodate the disk with no elongation or fold-

ing, no difference in result is noted. The more obvious explanation is that the developing bristles, crowded as they are within the small inner cavity of the implant which develops without everting, are subjected to a varying amount of compression and interference. The latter interpretation is supported by data which show that the largest bristles of the complement, *i.e.*, scutellars, dorso-centrals, anterior post-alar and posterior supra-alar, are most severely and most frequently affected. The number of bristles affected varies greatly. In a single implant from 10 to more than 50 per cent. of the larger bristles may exhibit some abnormality.

It is evident, therefore, that compression effects must be considered especially when studying the development of bristle-bearing tissues in such mutant hosts as forked and singed. The behavior of various mutant bristle characters on implantation into mutant and wild-type hosts is at present being studied.

ACKNOWLEDGMENT

Grateful acknowledgment is made to the American Philosophical Society for a grant-in-aid which greatly facilitated the progress of this work.

RUTH B. HOWLAND
E. A. GLANCY

WASHINGTON SQUARE COLLEGE,
NEW YORK UNIVERSITY

LITERATURE CITED

Ephrussi, B., and G. W. Beadle
1936. AM. NAT., 70: 218–225.
Chen, Tse-Yin
1929. *Jour. Morph. and Physiol.*, 47: 135–199.
Howland, R. B., B. P. Sonnenblick and E. A. Glancy
1937. AM. NAT., 71: 158–166.

NOTES ON THE HABITS OF THE TRIPLE-TAIL, *LOBOTES SURINAMENSIS*

SOME time ago, my attention was called to a paper[1] on the natural history of the triple-tail, written by Dr. E. W. Gudger, of the American Museum of Natural History. This I read with a great deal of interest and, encouraged by Dr. Gudger, I have prepared some notes which confirm and extend what he has written about this interesting fish. These notes come out of my personal observations or from fellow fishermen whom I know well.

[1] AMERICAN NATURALIST, 55: pp. 49–69, 5 figs., 1931.

The triple-tail has been seen in the summer sunning itself in Mosquito Inlet (Daytona) and at Fort Pierce (both on the east coast of Florida), but so far as I can find the only place on our east coast where the triple-tail has been taken is in Canaveral Harbor, just south of Point Canaveral. It is caught from early in May till the first cold weather in the fall, when it ceases to bite and seems to leave the harbor. Here it is known as the buoy-fish from its habit of gathering around buoys. But, in my experience, it is mainly caught from under shrimp boats anchored several hundred yards off shore. Often when a boat has been anchored only a few hours, it is possible to take from 10 to 25 triple-tails from under and around it. These run from 1.5 to 20 pounds in weight, with most of them between 3 and 15 pounds. A catch of 100 to 150 pounds is not unusual from under one boat, and by next day a new gathering is at hand, ready to be caught.

For any but an expert, a good 30-pound Cuttyhunk line is none too heavy. We fish in water from 3 to 8 feet deep and for bait use live or dead shrimp or shiners. This active fish does its own striking, and during its fight for freedom makes a number of short bulldog rushes of around 50 feet in length and generally comes to the surface two or three times.

How large the fish grows I do not know. My largest fish pulled 26 pounds on the scales and had a double roe weighing about 2 pounds. It measured 33 inches over all. Mr. Carl Prange and a companion, both of Orlando, in September, 1935, each hooked and landed triple-tails, which several hours later weighed 32 pounds each on the Canaveral fish pier scales. These fish were taken about 4.5 miles out by the wreck of a sunken steamer in about 50 feet of water. So far as I know these are the only ones taken from around this wreck.

So far as I know the triple-tail is taken only by hook and line or in nets. As it lies near the surface basking in the sun, it offers a fine target for the use of a gig or grains. But it has the toughest scales of any fish in our waters, and the gig glances off without penetrating the scales. So heavy are these scales that the only effective way of scaling a triple-tail is to take a heavy sharp knife and slice the scales off, beginning at the tail and working toward the head. But, caught, scaled, cleaned and either fried or baked, it is a fine-flavored food fish.

K. F. HUGHES

ORLANDO, FLA.